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(57) Abstract

The present invention relates to immunogenic Toxoplasma gondii proteins, to T. gondii nucleic acid molecules, including those that encode such proteins and to antibodies raised against such proteins. The present invention also includes methods to obtain such proteins, nucleic acid molecules and antibodies. Also included in the present invention are compositions comprising such proteins, nucleic acid molecules and/or antibodies, as well as the use of such compositions to inhibit oocyst shedding by cats due to infection with T. gondii. The present invention also includes the use of certain T. gondii-based antisera to identify such nucleic acid molecules and proteins, as well as nucleic acid molecules and proteins identified by such methods. The present invention also relates to methods for the detection of cysts and oocysts.

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TOXOPLASMA GONDII PROTEINS, CORRESPONDING NUCLEIC ACID MOLECULES, AND USES THEREOF

FIELD OF THE INVENTION

The present invention relates to *Toxoplasma gondii* nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins and methods to identify such nucleic acid molecules, proteins or antibodies. The present invention also includes compositions comprising such nucleic acid molecules, proteins and antibodies, as well as their use for inhibiting oocyst shedding by cats infected with *T. gondii* and for protecting animals from diseases caused by *T. gondii*.

BACKGROUND OF THE INVENTION

Various attempts to develop a vaccine to both the asexual systemic stage and the sexual entero-epithelial stage of the Toxoplasma life cycle have been reported over the last thirty years (Hermentin, K. and Aspock, H. (1988), Zbl. Bakt. Hyg. A, 269:423-436). These attempts can be grouped into the following categories: 1) immunization with whole killed organism, 2) immunization with selected antigens, either purified native or recombinant protein, 3) immunization with attenuated strains, and 4) immunization with irradiated organisms. Little success has been achieved with immunizations using whole killed organism (Frenkel, J.K. and Smith, D.D. (1982), Journal of Parasitology, 68:744-748). Partial success has been observed with the pure native protein P30 (Bulow, R., and Boothroyd, J. C. (1991), J. Immunol. 147:3496) and with selected fractions of parasite lysates (Lunden, A. Lovgren, K. Uggla, A., and Araujo, F.G.; (1993) Infection and Immunity, 61: 2639-2643). However, attempts with purified recombinant antigens have not been successful (Lunden, A., Parmley, S.F., Bengtsson, K.L. and Araujo, F.G. (1997) Parasitology Research, 83:6-9). Studies with irradiated organisms have reported 0-90% protection and are complicated by the uncertainty of truly inactivated irradiated preparations. Effective vaccines have been produced using attenuated strains. Two such mutant strains, ts-4 (Waldeland, H., Pfefferkorn, E.R., and Frenkel, J.K. (1983), Journal of Parasitology, 69:171-175) and S48 (Hartley, W.J. and Marshall, S.C. (1957), New Zealand Veterinary Journal, 5:119-124), successfully protect animals against the asexual systemic disease. These strains are delivered in the tachyzoite form and do not

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protect cats from oocyst shedding. Another strain, T-263 (Frenkel, J.K.; Pfefferkorn, E.R.; Smith, D.D.; and Fishback, J.L. (1991), American Journal of Veterinary Research, 52:759-763) is an oocyst minus strain, but was shown to progress through most of the entero-epithelial stages in the cat intestine. Exposure to this strain induces immunity in the cat to oocyst shedding upon subsequent challenge. There remains a need for an effective vaccine for prevention of the diseases caused by infection with Toxoplasma gondii.

SUMMARY OF THE INVENTION

The present invention relates to novel compositions and methods to inhibit *Toxoplasma gondii* (*T. gondii*) oocyst shedding by cats, thereby preventing the spread of *T. gondii* infection. According to the present invention there are provided isolated immunogenic *T. gondii* proteins and mimetopes thereof; *T. gondii* nucleic acid molecules, including those that encode such proteins; recombinant molecules including such nucleic acid molecules; recombinant viruses including such nucleic acid molecules; recombinant cells including such nucleic acid molecules; and antibodies that selectively bind to such immunogenic *T. gondii* proteins.

The present invention also includes methods to obtain and/or identify proteins, nucleic acid molecules, recombinant molecules, recombinant viruses, recombinant cells, and antibodies of the present invention. Also included are compositions comprising such proteins, nucleic acid molecules, recombinant molecules, recombinant viruses. recombinant cells, and antibodies, as well as use of such compositions to inhibit *T. gondii* oocyst shedding by cats infected with *T. gondii*, or for preventing *T. gondii* infection in an animal.

The present invention further includes the use of the nucleic acid molecules or proteins of the present invention as diagnostic reagents for the detection of *T. gondii* infection. In a preferred embodiment, the present invention includes a novel detection method and kit for detecting *T. gondii* oocysts in the feces of *T. gondii* infected cats.

One embodiment of the present invention is an isolated nucleic acid molecule encoding an immunogenic *T. gondii* protein that can be identified by a method that includes the steps of: a) immunoscreening a *T. gondii* genomic expression library or

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cDNA expression library with an antiserum, including an antiserum derived from intestinal secretions; and b) identifying a nucleic acid molecule in the library that expresses a protein that selectively binds to an antibody in the antiserum. Antisera to be used for screening include antiserum raised against *T. gondii* oocysts, antiserum raised against *T. gondii* infected cat gut, and antiserum isolated from a cat immune to *T. gondii* infection. Another embodiment is an isolated immunogenic *T. gondii* protein that can be identified by a method that includes the steps of: a) immunoscreening a *T. gondii* genomic expression library or cDNA expression library with such an antiserum; and b) identifying a protein expressed by the library that selectively binds to antibodies in the antiserum. Also included are methods to identify and isolate such nucleic acid molecules and proteins.

The present application also includes an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene that includes a nucleic acid sequence cited in Table 1. Also included in the present invention is an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene that includes a nucleic acid molecule cited in Table 1. Preferred nucleic acid molecules encode immunogenic *T. gondii* proteins. More preferred nucleic acid molecules are those cited in Table 1.

The present invention also relates to recombinant molecules, recombinant viruses and recombinant cells that include an isolated nucleic acid molecule of the present invention. Also included are methods to produce such nucleic acid molecules, recombinant molecules, recombinant viruses and recombinant cells.

Another embodiment of the present invention is an isolated immunogenic protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene (i.e., with either the coding strand or the non-coding strand) comprising a nucleic acid sequence cited in Table 1 and/or a nucleic acid molecule cited in Table 1. Note that the nucleic acid molecule hybridizes with the non-coding strand of the gene, that is, with the complement of the coding strand of the gene. A preferred protein is an immunogenic *T. gondii* protein. More preferred proteins are those encoded

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by nucleic acid molecules cited in Table 1. Also preferred are the proteins cited in Table 1.

The present invention also relates to: mimetopes of immunogenic *T. gondii* proteins and isolated antibodies that selectively bind to immunogenic *T. gondii* proteins or mimetopes thereof. Also included are methods, including recombinant methods, to produce proteins, mimetopes and antibodies of the present invention.

Yet another embodiment of the present invention is a composition to inhibit T. gondii oocyst shedding in a cat due to infection with T. gondii. Such a composition includes one or more of the following protective compounds: an isolated immunogenic T. gondii protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising a nucleic acid sequence cited in Table 1, and specifically with the non-coding-strand of that gene; an isolated antibody that selectively binds to said immunogenic T. gondii protein; and an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising a nucleic acid sequence cited in Table 1. Such a composition can also include an excipient, adjuvant or carrier. Preferred compositions comprising a nucleic acid molecule of the present invention include genetic vaccines, recombinant virus vaccines and recombinant cell vaccines. Also included in the present invention is a method to protect an animal, including a human, from disease caused by T. gondii, comprising the step of administering to the animal a composition of the present invention. Preferred animals to treat are cats in order to prevent oocyst shedding caused by T. gondii infection.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides for isolated immunogenic *T. gondii* proteins, isolated *T. gondii* nucleic acid molecules including those encoding such *T. gondii* proteins, recombinant molecules comprising such nucleic acid molecules, recombinant viruses comprising such nucleic acid molecules, cells transformed with such nucleic acid molecules (i.e., recombinant cells), and antibodies that selectively bind to immunogenic *T. gondii* proteins. As used herein, the terms isolated immunogenic *T. gondii* protein and isolated nucleic acid molecule refer to an immunogenic *T. gondii* protein and a *T. gondii* nucleic acid molecule, respectively, derived from *T. gondii* which can be obtained from its natural source or can be produced using, for example, recombinant nucleic acid

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technology or chemical synthesis. Also included in the present invention is the use of these proteins, nucleic acid molecules, and antibodies as compositions to protect animals from diseases caused by *T. gondii* and to inhibit *T. gondii* oocyst shedding in cats. As used herein, a cat refers to any member of the cat family (i.e., Felidae), including domestic cats, wild cats and zoo cats. Examples of cats include, but are not limited to, domestic cats, lions, tigers, leopards, panthers, cougars, bobcats, lynx, jaguars, cheetahs, and servals. A preferred cat to protect is a domestic cat. Further included in the present invention is the use of these proteins, nucleic acid molecules and antibodies for the detection of *T. gondii* infection in an animal or as targets for the development of chemotherapeutic agents against parasitic infection.

Immunogenic *T. gondii* protein and nucleic acid molecules of the present invention have utility because they represent novel targets for anti-parasite vaccines or chemotherapeutic agents. Compositions of the present invention can also be used as reagents for the diagnosis of *T. gondii* infection in cats and other animals, including humans. The products and processes of the present invention are advantageous because they enable the inhibition of *T. gondii* oocyst shedding in cats, the definitive hosts for *T. gondii* (i.e., the animals in which *T. gondii* reproduction takes place). It is to be noted that the proteins and nucleic acid molecules of the present invention have uses beyond eliciting an immune response despite denoting proteins of the present invention as immunogenic proteins.

As described in more detail in the Examples, it was very difficult to isolate a nucleic acid molecule encoding an immunogenic T. gondii protein selectively bound by antisera directed against T. gondii intestinal stages. Such stages are preterred because they represent the sexual cycle of T. gondii, the preferred target for development of a composition to inhibit oocyst shedding. Unfortunately, however, the T gondii sexual cycle cannot currently be reproduced in culture, and, there is not a simple method by which to produce a cDNA (i.e., complementary DNA) library containing only T gondii nucleic acid molecules of various stages of the sexual cycle. For example, the infected cat gut is the source of many of the sexual stages of T gondii, and, as such, material to be used in identifying T. gondii immunogenic proteins are contaminated with cat

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material. The present invention describes the development of new techniques to isolate and identify nucleic acid molecules encoding immunogenic *T. gondii* proteins. These techniques include (a) the isolation and enrichment of antisera against a variety of *T. gondii* life stages, several of which are only present in infected cats, at least predominantly in infected cat guts, and (b) the use of such antisera to screen cDNA and genomic expression libraries to identify nucleic acid molecules that express *T. gondii* proteins that selectively bind to such antisera.

One embodiment of the present invention is an isolated protein that includes an immunogenic *T. gondii* protein. It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, a protein refers to one or more proteins or at least one protein. The terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein. It is also to be noted that the terms "comprising", "including", and "having" can be used interchangeably. According to the present invention, an isolated, or biologically pure, protein is a protein that has been removed from its natural milieu. The terms "isolated" and "biologically pure" do not necessarily reflect the extent to which the protein has been purified. An isolated protein of the present invention can be obtained from its natural source, can be produced using recombinant DNA technology, or can be produced by chemical synthesis.

An isolated protein of the present invention, including a homolog, can be identified in a straight-forward manner by the protein's ability to elicit an immune response against a naturally occurring *T. gondii* protein. Examples of *T. gondii* immunogenic proteins include proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitoylation, amidation and/or addition of glycerophosphatidyl inositol) such that the homolog includes at least one epitope capable of eliciting an immune response against a *T. gondii* immunogenic protein, and/or of binding to an antibody directed against a *T. gondii* immunogenic protein. That is, when the homolog is administered to an animal as an immunogen, using techniques known to those skilled in the art, the animal will produce an immune response against at least one epitope of a *T.*

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be measured using techniques known to those skilled in the art. As used herein, the term "epitope" refers to the smallest portion of a protein or other antigen capable of selectively binding to the antigen binding site of an antibody or a T-cell receptor. It is well accepted by those skilled in the art that the minimal size of a protein epitope is about four to six amino acids. As is appreciated by those skilled in the art, an epitope can include amino acids that naturally are contiguous to each other as well as amino acids that, due to the tertiary structure of the natural protein, are in sufficiently close proximity to form an epitope. According to the present invention, an epitope includes a portion of a protein comprising at least about 4 amino acids, at least about 5 amino acids, at least about 6 amino acids, at least about 10 amino acids, at least about 30 amino acids, at least about 35 amino acids, at least about 40 amino acids, at least about 50 amino acids, at least about 100 amino acids, at least about 30 amino acids, at least about 200 amino acids, at least about 250 amino acids, at least about 300 amino acids.

Immunogenic *T. gondii* protein homologs can be the result of natural allelic variation or natural mutation. Immunogenic *T. gondii* protein homologs of the present invention can also be produced using techniques known in the art including, but not limited to, direct modifications to the protein or modifications to the gene encoding the protein using, for example, classic or recombinant DNA techniques to effect random or targeted mutagenesis.

As used herein, a nucleic acid molecule encoding an immunogenic *T. gondii* protein includes nucleic acid sequences related to a natural *T. gondii* gene. As used herein, a *T. gondii* gene includes all regions of the genome related to the gene, such as regulatory regions that control production of the immunogenic *T. gondii* protein encoded by the gene (for example, transcription, translation or post-translation control regions) as well as the coding region itself, and any introns or non-translated coding regions. As used herein, a gene that "includes" or "comprises" a sequence may include that sequence in one contiguous array, or may include the sequence as fragmented exons. As used herein, the term "coding region" refers to a continuous linear array of nucleotides that

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translates into a protein. A full-length coding region is that coding region that is translated into a full-length protein, i.e., a complete protein as would be initially translated in its natural milieu, prior to any post-translational modifications.

In one embodiment, a *T. gondii* gene of the present invention includes at least one of the nucleic acid molecules cited in Table 1 (i.e., the cited nucleic acid molecules). The coding strands of the cited nucleic acid molecules are represented, respectively, by the nucleic acid sequences (i.e., the cited nucleic acid sequences) shown in Table 1. Also presented in Table 1 are the deduced amino acid sequences encoded by each of the cited nucleic acid molecules (i.e., the cited amino acid sequences) and the protein name designations (i.e., the cited proteins).

		Nucleic Acid	Amino Acid	
SEQ ID NO	TYPE	Molecules	Molecules	Original Designation
4	: 	· 	<u> </u>	
1	DNA	nTG1 ₃₅₇		Tg-41
2	Protein		PTG1 ₁₁₉	PTG-41
3	DNA	nTG2 ₃₃₉	<u> </u>	Tg-45
4	Protein		PTG2 ₁₀₈	PTG-45
5	DNA	nTG4 ₅₂₆		Tg-50
6	Protein		PTG4 ₁₇₅	PTG-50
7	cDNA	nTG4 ₁₄₇₈		Tg-50c
8	Protein		PTG4 ₃₈₁	PTG-50c
9	DNA	nTG5 ₆₅₇		Q2-4
10	Protein		PTG5 ₂₁₉	PQ2-4
11	cDNA	nTG5 ₁₀₂₉		Q2-4c
12	Protein		PTG5 ₂₇₃	PQ2-4c
13	DNA	nTG6 ₄₂₅		Q2-9
14	Protein		PTG6 ₁₄₂	PQ2-9
15	DNA	nTG7 ₄₁₇		Q2-10
16	Protein		PTG7 ₁₃₉	PQ2-10
17	DNA	nTG8 ₅₀₇		Q2-11
18	Protein		PTG8 ₅₁	PQ2-11
19	DNA	nTG9 ₇₁₈		4499-9
20	Protein		PTG9 ₉₉	P4499-9
21	DNA	nTG10 ₄₄₁		4604-2
22	Protein		PTG.10 ₁₄₇	P4604-2
23	DNA	nTG11 ₄₂₈		4604-3
24	Protein		PTG11 ₁₃₄	P4604-3
25	DNA	nTG13 ₂₈₂		4604-5
26	DNA	nTG15 ₃₀₄		4604-10
27	Protein		PTG15 ₁₀₁	P4604-10
28	DNA	nTG16 ₂₈₄		4604-17
29	Protein		PTG16 ₉₅	P4604-17
30	DNA	nTG17 ₆₉₀		4604-54
31	Protein		PTG17 ₂₃₀	P4604-54
32	DNA	nTG18 ₃₁₃		4604-62
33	Protein		PTG18 ₅₄	P4604-62
34	DNA	nTG19 ₃₈₉		4604-63
35	Protein		PTG19 ₆₅	P4604-63

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SEQ ID NO	TYPE	Nucleic Acid Molecules	Amino Acid Molecules	Original Designation
36	DNA	nTG21 ₅₄₈		4604-69
37	Protein		PTG21 ₁₈₃	P4604-69
38	DNA	nTG22 ₃₁₀		BZ1-2
39	Protein		PTG22 ₉₅	PBZ1-2
40	DNA	nTG23 ₂₂₀		BZ1-3
41	Protein		PTG23 ₇₃	PBZ1-3
42	DNA	nTG24 ₆₄₂		BZ1-6
43	Protein		PTG24 ₃₄	PBZ1-6
44	DNA	nTG25 ₃₈₁		BZ2-3
45	Protein		PTG25 ₂₇	PBZ2-3
46	DNA	nTG26 ₄₃₂		BZ2-5
47	Protein		PTG26 ₈₅	PBZ2-5
48	DNA	nTG27 ₂₈₂		BZ3-2
49	Protein		PTG27 ₃₅	PBZ3-2
50	DNA	nTG28 ₄₆₆		BZ4-3
51	Protein		PTG28 ₇₁	PBZ4-3
52	DNA	nTG30 ₅₃₉		BZ4-6
53	Protein		PTG30 ₂₀	PBZ4-6
54	DNA r	nTG31 ₁₂₃₃		AMX/I-5
55	DNA r	nTG32 ₄₁₁		AMX/I-6
56	Protein		PTG32 ₆₀	PAMX/I-6
57	DNA r	nTG33 ₄₄₁		AMX/I-7
58	Protein		PTG33 ₁₁₈	PAMX/I-7
59	DNA r	TG34 ₄₉₁		AMX/I-9
60	Protein			PAMX/I-9
61	DNA n	TG35 ₃₈₇		AMX/I-10
62	Protein			PAMX/I-10
63	DNA n	TG36 ₄₁₇		AMI-23
64	Protein			PAMI-23
65	DNA n	TG37 ₄₁₆		AMI-24
66	Protein			PAMI-24
67	DNA n	TG38 ₅₀₀		AMI-28
68		TG40 ₃₂₁		AMI-47
69	Protein			PAMI-47
70	DNA n	TG41 _{513+C86}		OC-1
71	Protein			POC-1

Table 1

SEQ ID NO	TYPE	Nucleic Acid	Amino Acid	0-1-1-1-1
SEC ID NO	ITPE	Molecules	Molecules	Original Designation
70	5214	TO 40	<u>:</u>	
72	DNA	nTG42 ₅₂₈	 	OC-2
73	Protein		PTG42 ₁₇₆	POC-2
74	DNA	nTG43 ₃₇₅		OC-13
75	Protein		PTG43 ₁₂₅	POC-13
76	DNA	nTG44 ₅₄₃		OC-14
77	Protein		PTG44 ₈₉	POC-14
78	DNA	nTG45 ₅₇₃		OC-22
79	Protein		PTG45 ₁₉₁	POC-22
80	DNA	nTG46 ₁₈₃₅		OC-23
81	Protein		PTG46 ₆₁₂	POC-23
82	DNA	nTG48 ₆₀₄		4CQA7f
83	Protein		PTG48 ₁₁₂	P4CQA7f
84	DNA	nTG48 ₅₄₉		4CQA7r
85	DNA	nTG49 ₂₇₀		4CQA11
86	Protein		PTG49 ₉₀	P4CQA11
87	DNA	nTG50 ₃₀₆		4CQA19
88	Protein		PTG50 ₁₀₂	P4CQA19
89	DNA	nTG51 ₈₀₄		4CQA21
90	Protein		PTG51 ₂₆₈	P4CQA21
91	DNA	nTG52 ₈₆₇		4CQA22
92	Protein		PTG52 ₂₈₉	P4CQA22
93	DNA	nTG53 ₁₄₃₄		4CQA24
94	Protein		PTG53 ₁₆₄	P4CQA24
95	DNA	nTG54 ₆₈₀		4CQA25
96	Protein		PTG54 ₂₂₇	P4CQA25
97	DNA	nTG55 ₂₉₆		4CQA26
98	Protein		PTG55 ₉₉	P4CQA26
99	DNA	nTG56 ₇₂₃		4CQA27
100	Protein		PTG56 ₅₃	P4CQA27
101	DNA	nTG57 ₂₇₀		4CQA29
102	Protein		PTG57 ₉₀	P4CQA29
103	DNA	nTG58 ₅₀₃		R8050-2
104	Protein		PTG58 ₆₂	PR8050-2
105	DNA	nTG60 ₃₂₂		R8050-5
106	Protein		PTG60 ₇₃	PR8050-5

SEQ ID NO	TYPE	Nucleic Acid Molecules	Amino Acid Molecules	Original Designation
	:			- Chighiai Designation
107	DNA	nTG61 ₃₉₀		R8050-6
108	Protein		PTG61 ₆₇	PR8050-6
109	DNA	nTG62 ₆₉₉	 	M2A1
110	Protein		PTG62 ₂₃₃	PM2A1
111	DNA	nTG63 ₄₁₉		M2A2
112	Protein		PTG63 ₁₄₀	PM2A2
113	DNA	nTG64 ₃₀₃		M2A3
114	Protein		PTG64 ₁₀₁	PM2A3
115	DNA	nTG65 ₆₉₆	107	M2A4
116	Protein		PTG65 ₂₃₂	PM2A4
117	DNA	nTG66 ₁₇₃	252	M2A5
118	Protein		PTG66 ₅₈	PM2A5
119	DNA	nTG67 ₃₆₉	30	M2A6
120	Protein	<u> </u>	PTG67 ₁₂₃	PM2A6
121	DNA	nTG68 ₅₆₆	123	M2A7
122	Protein		PTG68 ₆₁	PM2A7
123	DNA	nTG69 ₆₁₆		M2A11
124	Protein		PTG69 ₂₀₅	PM2A11
125	DNA	nTG70 ₇₆₂	203	M2A16
126	Protein		PTG70 ₂₅₄	PM2A16
127	DNA	nTG71 ₂₃₆		M2A18
128	Protein		PTG71 ₇₉	PM2A18
129	DNA	nTG72 ₅₆₉		M2A19
130	Protein		PTG72 ₁₉₀	PM2A19
131	DNA	nTG73 ₂₃₂		M2A20
132	DNA	nTG74 ₂₇₆	· · · · · · · · · · · · · · · · · · ·	M2A21
133	Protein		PTG74 ₉₂	PM2A21
134	DNA	nTG75 ₃₀₉		M2A22
135	Protein			PM2A22
136	DNA I	nTG76 ₅₃₄		M2A23
137	Protein			PM2A23
138	DNA r	TG76 ₄₂₃		M2A23
139	DNA r	TG77 ₃₂₇		M2A24
140	Protein		PTG77 ₁₀₉	PM2A24
141	DNA r	TG78 ₄₄₄		M2A25
142	Protein			PM2A25

		Nucleic Acid	Amino Acid	
SEQ ID NO	TYPE	Molecules	Molecules	Original Designation
143	DNA	nTG79 ₉₂₈	1	M2A29
144	Protein	0. 0928	PTG79 ₁₉	PM2A29
265	DNA	nTG22 _{310a}	13	BZ1-2-a
266	Protein		PTG22 _{95a}	PBZ1-2-a
267	DNA	nTG64 _{303a}		M2A3-a
268	Protein		PTG64 _{101a}	PM2A3-a
269	DNA	nTG71 _{236a}		M2A18-a
270	Protein		PTG71 _{79a}	PM2A18-a
271	DNA	nTG6 _{425a}		Q2-9-1-a
272	Protein		PTG6 _{142a}	PQ2-9-a
273	DNA	nTG41 _{513a}		OC-1-a
274	Protein		PTG41 _{171a}	POC-1-a
282	cDNA	nTG ₁₂₂₅		MGIS42
283	Protein		PTG ₂₈	PMGIS42
284	DNA	nTG ₁₂₂₅		rc
292	cDNA	nTG ₁₅₇₃		MGIS44
293	Protein		PTG ₇₃	PMGIS44
294	DNA	nTG ₁₅₇₃		rc
306	cDNA	nTG ₂₄₁₇		MGIS48
307	Protein		PTG ₉	PMGIS48
308	DNA	nTG ₂₄₁₇		rc
311	cDNA	nTG ₁₇₈₅		MGIS65
312	Protein		PTG ₂₄	PMGIS65
313	DNA	nTG ₁₇₈₅		rc
338	DNA	nTG ₆₄₇		511-44 genomic
339	DNA	nTG ₆₄₇		rc
340	cDNA	nTG ₈₆₇		511-44 coding region
341	Protein		PTG ₂₈₈	P511-44
342	DNA	nTG ₈₆₇		rc
343	cDNA	nTG ₁₃₉₇		511-44cDNA
345	DNA	nTG ₁₃₉₇		rc

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It should be noted that because nucleic acid sequencing technology is not entirely error-free, the nucleic acid sequences disclosed in the present invention (as well as other nucleic acid and protein sequences presented herein) represent the apparent nucleic acid sequences of the nucleic acid molecules encoding *T. gondii* proteins of the present invention. The nucleic acid molecules cited in Table 1 also include the complementary (i.e., apparently non-coding) strands. As used herein the terms "complementary strand" and "complement" refer to the nucleic acid sequence of the DNA strand that is fully complementary to the DNA strand having the listed sequence, which can easily be determined by those skilled in the art. Likewise, a nucleic acid sequence complement of any nucleic acid sequence of the present invention refers to the nucleic acid sequence of the nucleic acid strand that is fully complementary to (i.e., can form a complete double helix with) the strand for which the sequence is cited. Production of the cited nucleic acid molecules is disclosed in the Examples as are methods to obtain nucleic acid sequences of the coding strands of such molecules and the amino acid sequences deduced therefrom.

In another embodiment, a T. gondii gene or nucleic acid molecule can be a naturally occurring allelic variant that includes a similar but not identical sequence to the cited nucleic acid molecules. A naturally occurring allelic variant of a T. gondii gene including any of the above-listed nucleic acid sequences is a gene that occurs at essentially the same locus (or loci) in the genome as the gene including at least one of the above-listed sequences, but which, due to natural variations caused by, for example, mutation or recombination, has a similar but not identical sequence. Because natural selection typically selects against alterations that affect function, allelic variants usually encode proteins having similar activity to that of the protein encoded by the gene to which they are being compared. Allelic variants of genes or nucleic acid molecules can also comprise alterations in the 5' or 3' untranslated regions of the gene (e.g., in regulatory control regions), or can involve alternative splicing of a nascent transcript, thereby bringing alternative exons into juxtaposition. Allelic variants are well known to those skilled in the art and would be expected to be found within a given T. gondii organism or population, because, for example, the genome goes through a diploid stage, and sexual reproduction results in the reassortment of alleles.

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In one embodiment of the present invention, an isolated immunogenic *T. gondii* protein is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions to a gene encoding an immunogenic *T. gondii* protein. The minimal size of a *T. gondii* protein of the present invention is a size sufficient to be encoded by a nucleic acid molecule capable of forming a stable hybrid (i.e., hybridizing under stringent hybridization conditions) with the complementary sequence of a nucleic acid molecule encoding the corresponding natural protein. The size of a nucleic acid molecule encoding such a protein is dependent on the nucleic acid composition and the percent homology between the *T. gondii* nucleic acid molecule and the complementary nucleic acid sequence. It can easily be understood that the extent of homology required to form a stable hybrid under stringent conditions can vary depending on whether the homologous sequences are interspersed throughout a given nucleic acid molecule or are clustered (i.e., localized) in distinct regions on a given nucleic acid molecule.

The minimal size of a nucleic acid molecule capable of forming a stable hybrid with a gene encoding an immunogenic T. gondii protein is typically at least about 12 to about 15 nucleotides in length if the nucleic acid molecule is GC-rich and at least about 15 to about 17 bases in length if it is AT-rich. The minimal size of a nucleic acid molecule used to encode an immunogenic T. gondii protein homolog of the present invention is from about 12 to about 18 nucleotides in length. Thus, the minimal size of an immunogenic T. gondii protein homolog of the present invention is from about 4 to about 6 amino acids in length. There is no limit, other than a practical limit, on the maximal size of a nucleic acid molecule encoding an immunogenic T. gondii protein of the present invention because a nucleic acid molecule of the present invention can include a portion of a gene, an entire gene, or multiple genes. A preferred nucleic acid molecule of the present invention is a nucleic acid molecule that is at least 12 nucleotides in length. Also preferred are nucleic acid molecules that are at least 18 nucleotides, or at least 20 nucleotides, or at least 25 nucleotides, or at least 30 nucleotides, or at least 40 nucleotides, or at least 50 nucleotides, or at least 70 nucleotides, or at least 100 nucleotides, or at least 150 nucleotides, or at least 200 nucleotides, or at least 250 nucleotides, or at least 300 nucleotides, or at least 350 nucleotides, or at least 400 nucleotides, or at least 500 nucleotides, or at least 750

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nucleotides, or at least 1000 nucleotides, or at least 1500 nucleotides, or at least 1750 nucleotides, or at least 2000 nucleotides, or at least 2250 nucleotides, or at least 2417 nucleotides in length, The preferred size of a protein encoded by a nucleic acid molecule of the present invention depends on whether a full-length, fusion, multivalent, or functional portion of such a protein is desired.

Stringent hybridization conditions are determined based on defined physical properties of the gene to which the nucleic acid molecule is being hybridized, and can be defined mathematically. Stringent hybridization conditions are those experimental parameters that allow an individual skilled in the art to identify significant similarities between heterologous nucleic acid molecules. These conditions are well known to those 10 skilled in the art. See, for example, Sambrook, et al., 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Labs Press, and Meinkoth, et al., 1984, Anal. Biochem. 138, 267-284, each of which is incorporated by reference herein in its entirety. As explained in detail in the cited references, the determination of hybridization conditions involves the manipulation of a set of variables including the ionic strength (M, in moles/liter), the hybridization temperature (°C), the concentration of nucleic acid helix destabilizing agents (such as formamide), the average length of the shortest hybrid duplex (n), and the percent G + C composition of the fragment to which an unknown nucleic acid molecule is being hybridized. For nucleic acid molecules of at least about 150 nucleotides, these variables are inserted into a standard mathematical formula to calculate the melting temperature, or T_m, of a given nucleic acid molecule. As defined in the formula below, T_m is the temperature at which two complementary nucleic acid molecule strands will disassociate, assuming 100% complementarity between the two strands:

 T_m =81.5°C + 16.6 log M + 0.41(%G + C) - 500/n - 0.61(%formamide). 25 For nucleic acid molecules smaller than about 50 nucleotides, hybrid stability is defined by the dissociation temperature (T_d), which is defined as the temperature at which 50% of the duplexes dissociate. For these smaller molecules, the stability at a standard ionic strength is defined by the following equation:

$$T_d = 4(G+C) + 2(A+T).$$

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A temperature of 5°C below T_d is used to detect hybridization between perfectly matched molecules.

Also well known to those skilled in the art is how base-pair mismatch, i.e. differences between two nucleic acid molecules being compared, including noncomplementarity of bases at a given location, and gaps due to insertion or deletion of one or more bases at a given location on either of the nucleic acid molecules being compared, will affect T_m or T_d for nucleic acid molecules of different sizes. For example, T_m decreases about 1°C for each 1% of mismatched base-pairs for hybrids greater than about 150 bp, and T_d decreases about 5°C for each mismatched base-pair for hybrids below about 50 bp. Conditions for hybrids between about 50 and about 150 base-pairs can be determined empirically and without undue experimentation using standard laboratory procedures well known to those skilled in the art. These simple procedures allow one skilled in the art to set the hybridization conditions (by altering, for example, the salt concentration, the formamide concentration or the temperature) so that only nucleic acid hybrids with less than a specified % base-pair mismatch will hybridize. Stringent hybridization conditions are commonly understood by those skilled in the art to be those experimental conditions that will allow hybridization between molecules having about 30% or less base-pair mismatch (i.e., about 70% or greater identity). Because one skilled in the art can easily determine whether a given nucleic acid molecule to be tested is less than or greater than about 50 nucleotides, and can therefore choose the appropriate formula for determining hybridization conditions, he or she can determine whether the nucleic acid molecule will hybridize with a given gene under stringent hybridization conditions and similarly whether the nucleic acid molecule will hybridize under conditions designed to allow a desired amount of base pair mismatch.

Hybridization reactions are often carried out by attaching the nucleic acid molecule to be hybridized to a solid support such as a membrane, and then hybridizing with a labeled nucleic acid molecule, typically referred to as a probe, suspended in a hybridization solution. Examples of common hybridization reaction techniques include, but are not limited to, the well-known Southern and northern blotting procedures.

30 Typically, the actual hybridization reaction is done under non-stringent conditions, i.e.,

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at a lower temperature and/or a higher salt concentration, and then high stringency is achieved by washing the membrane in a solution with a higher temperature and/or lower salt concentration in order to achieve the desired stringency.

For example, if the skilled artisan wished to identify a nucleic acid molecule that hybridizes under stringent hybridization conditions with a *T. gondii* nucleic acid molecule of about 150 bp in length, the following conditions could preferably be used. As an example, the average G + C content of *Dirofilaria immitis* DNA is about 35%. The unknown nucleic acid molecules would be attached to a support membrane, and the 150 bp probe would be labeled, e.g. with a radioactive tag. The hybridization reaction could be carried out in a solution comprising 2X SSC and 0% formamide, at a temperature of about 37°C (low stringency conditions). Solutions of differing concentrations of SSC can be made by one of skill in the art by diluting a stock solution of 20X SSC (175.3 gram NaCl and about 88.2 gram sodium citrate in 1 liter of water, pH 7) to obtain the desired concentration of SSC. In order to achieve high stringency hybridization, the skilled artisan would calculate the washing conditions required to allow up to 30% base-pair mismatch. For example, in a wash solution comprising 1X SSC and 0% formamide, the T_m of perfect hybrids would be about 79°C:

 $81.5^{\circ}\text{C} + 16.6 \log (.15\text{M}) + (0.41 \times 35) - (500/150) - (0.61 \times 0) = 79^{\circ}\text{C}.$

Thus, to achieve hybridization with nucleic acid molecules having about 30% base-pair mismatch, hybridization washes would be carried out at a temperature of about 49°C. It is thus within the skill of one in the art to calculate additional hybridization temperatures based on the desired percentage base-pair mismatch, formulae and G/C content disclosed herein. For example, it is appreciated by one skilled in the art that as the nucleic acid molecule to be tested for hybridization against nucleic acid molecules of the present invention having sequences specified herein becomes longer than 150 nucleotides, the T_m for a hybridization reaction allowing up to 30% base-pair mismatch will not vary significantly from 49°C.

Furthermore, it is known in the art that there are commercially available computer programs for determining the degree of similarity between two nucleic acid sequences. These computer programs include various known methods to determine the

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percentage identity and the number and length of gaps between hybrid nucleic acid molecules. Preferred methods to determine the percent identity among amino acid sequences and also among nucleic acid sequences include analysis using one or more of the commercially available computer programs designed to compare and analyze nucleic acid or amino acid sequences. These computer programs include, but are not limited to, GCGTM (available from Genetics Computer Group, Madison, WI), DNAsisTM (available from Hitachi Software, San Bruno, CA) and MacVectorTM (available from the Eastman Kodak Company, New Haven, CT). A preferred method to determine percent identity among amino acid sequences and also among nucleic acid sequences includes using the GCGTM program, Bestfit function with default parameter settings, or a gap weight of 12, a length weight of 4, an average match of 2.912, and an average mismatch of -2.003.

A preferred immunogenic *T. gondii* protein of the present invention is a compound that, when administered to an animal in an effective manner, is capable of protecting that animal from disease caused by *T. gondii* or, in the case of cats, is capable of preventing *T. gondii* oocyst shedding in cats infected with *T. gondii*. In accordance with the present invention, the ability of an immunogenic *T. gondii* protein of the present invention to protect an animal from *T. gondii* disease refers to the ability of that protein to, for example, treat, ameliorate and/or prevent disease caused by *T. gondii*. In one embodiment, an immunogenic *T. gondii* protein of the present invention can elicit an immune response (including a humoral and/or cellular immune response) against *T. gondii*.

The present invention also includes mimetopes of immunogenic *T. gondii* proteins of the present invention. As used herein, a mimetope of an immunogenic *T. gondii* protein of the present invention refers to any compound that is able to mimic the activity of such an immunogenic *T. gondii* protein, often because the mimetope has a structure that mimics the particular *T. gondii* protein. Mimetopes can be, but are not limited to: peptides that have been modified to decrease their susceptibility to degradation such as all-D retro peptides; anti-idiotypic and/or catalytic antibodies, or fragments thereof; non-proteinaceous immunogenic portions of an isolated protein (e.g., carbohydrate structures); and synthetic or natural organic molecules, including nucleic

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acids. Such mimetopes can be designed using computer-generated structures of proteins of the present invention. Mimetopes can also be obtained by generating random samples of molecules, such as oligonucleotides, peptides or other organic molecules, and screening such samples by affinity chromatography techniques using the corresponding binding partner.

One embodiment of an immunogenic T. gondii protein of the present invention is a fusion protein that includes an immunogenic T. gondii protein-containing domain attached to one or more fusion segments. Suitable fusion segments for use with the present invention include, but are not limited to, segments that can: enhance a protein's stability; act as an immunopotentiator to enhance an immune response against an 10 immunogenic T. gondii protein; and/or assist in purification of an immunogenic T. gondii protein (e.g., by affinity chromatography). A suitable fusion segment can be a domain of any size that has the desired function (e.g., imparts increased stability, imparts increased immunogenicity to a protein, and/or simplifies purification of a protein). Fusion segments can be joined to amino and/or carboxyl termini of the immunogenic T. 15 gondii protein-containing domain of the protein and can be susceptible to cleavage in order to enable straightforward recovery of an immunogenic T. gondii protein. Fusion proteins are preferably produced by culturing a recombinant cell transformed with a nucleic acid molecule that encodes a protein including the fusion segment attached to either the carboxyl and/or amino terminal end of an immunogenic T. gondii protein-20 containing domain. Preferred fusion segments include a metal binding domain (e.g., a poly-histidine segment); an immunoglobulin binding domain (e.g., Protein A; Protein G; T cell; B cell; Fc receptor or complement protein antibody-binding domains); a sugar binding domain (e.g., a maltose binding domain); and/or a "tag" domain (e.g., at least a portion of β-galactosidase, a strep tag peptide, a T7 tag peptide, a FlagTM peptide, or 25 other domains that can be purified using compounds that bind to the domain, such as monoclonal antibodies). More preferred fusion segments include metal binding domains, such as a poly-histidine segment; a maltose binding domain: a strep tag peptide, such as that available from Biometra in Tampa, FL; and an S10 peptide. 30

In another embodiment, an immunogenic *T. gondii* protein of the present invention also includes at least one additional protein segment that is capable of

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protecting an animal from one or more diseases. Such a multivalent protective protein can be produced, for example, by culturing a cell transformed with a nucleic acid molecule comprising two or more nucleic acid domains joined together in such a manner that the resulting nucleic acid molecule is expressed as a multivalent protective compound containing at least two protective compounds capable of protecting an animal from diseases caused, for example, by at least one infectious agent.

Examples of multivalent protective compounds include, but are not limited to, an immunogenic *T. gondii* protein of the present invention attached to one or more compounds protective against one or more other infectious agents, particularly an agent that infects cats. In another embodiment, one or more protective compounds can be included in a multivalent vaccine comprising an immunogenic *T. gondii* protein of the present invention and one or more other protective molecules as separate compounds.

A preferred isolated immunogenic *T. gondii* protein of the present invention includes a protein that is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene (i.e., with the non-coding strand which is a complement of the coding strand) comprising at least one of the nucleic acid molecules cited in Table 1. As such, also preferred is a protein that is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with the non-coding strand of a gene comprising at least one of the nucleic acid sequences cited in Table 1. More preferred is a protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with at least one of the cited nucleic acid molecules particularly since those nucleic acid molecules have been shown to encode proteins that selectively bind to antiserum that either was raised against *T. gondii* oocysts, bradyzoites, or infected cat gut, or was isolated from a cat immune to *T. gondii* infection. As such, also preferred is a protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with the complement of at least one of the cited nucleic acid sequences.

Even more preferred are isolated proteins having an amino acid sequence encoded by a nucleic acid molecules that are at least about 75%, preferably at least about 80%, more preferably at least about 85%, even more preferably at least about 90%, even more preferably at least about 95%, and even more preferably at least about 98%

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identical to one of the nucleic acid molecules and/or nucleic acid sequences cited in Table 1. Also preferred are proteins that comprise one or more epitopes of any of the proteins having such amino acid sequences.

A particularly preferred isolated protein of the present invention is a protein having an amino acid requence encoded by at least one of the cited nucleic acid molecules and/or cited nucleic acid sequences, a protein encoded by an allelic variant of at least one of the cited nucleic acid molecules and/or nucleic acid sequences, or a protein comprising an epitope of any of the proteins having such amino acid sequences.

In one embodiment, preferred immunogenic *T. gondii* proteins of the present invention include proteins that are at least about 75%, preferably at least about 80%, more preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to at least one of the proteins cited in Table 1. As such, also preferred are proteins that are at least about 75%, preferably at least about 80%, more preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to at least one of the amino acid sequences cited in Table 1. Also preferred are proteins that comprise one or more epitopes of any of such proteins. More preferred are immunogenic *T. gondii* proteins comprising the cited proteins and/or having the cited amino acid sequences, proteins encoded by allelic variants of nucleic acid molecules encoding proteins including the cited proteins and/or having the cited amino acid sequences, and proteins having one or more epitopes of such proteins.

Another embodiment of the present invention is an isolated nucleic acid molecule comprising a *T. gondii* nucleic acid molecule that encodes an immunogenic *T. gondii* protein. The identifying characteristics of such nucleic acid molecules are heretofore described. A nucleic acid molecule of the present invention can include an isolated natural *T. gondii* nucleic acid molecule or a homolog thereof, the latter of which is described in more detail below. A nucleic acid molecule of the present invention can include one or more regulatory regions, full-length or partial coding regions, or combinations thereof. The minimal size of a nucleic acid molecule of the present invention is a size sufficient to allow the formation of a stable hybrid (i.e., hybridization under stringent hybridization conditions) with the complementary sequence of another

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nucleic acid molecule. The minimal size of an *T. gondii* nucleic acid molecule of the present invention is from about 12 to about 18 nucleotides in length.

In accordance with the present invention, an isolated nucleic acid molecule is a nucleic acid molecule that has been removed from its natural milieu (i.e., that has been subjected to human manipulation) and can include DNA, RNA, or derivatives of either DNA or RNA. Accordingly, the term "isolated", as used herein to describe a nucleic acid molecule, does not reflect the extent to which the nucleic acid molecule has been purified. An isolated *T. gondii* nucleic acid molecule of the present invention can be isolated from its natural source or produced using recombinant nucleic acid technology (e.g., polymerase chain reaction (PCR) amplification or cloning) or chemical synthesis. Isolated *T. gondii* nucleic acid molecules can include, for example, natural allelic variants and nucleic acid molecules modified by nucleotide insertions, deletions, substitutions, and/or inversions in a manner such that the modifications do not substantially interfere with the nucleic acid molecule's ability to encode an immunogenic *T. gondii* protein of the present invention.

A homolog of a nucleic acid molecule encoding an immunogenic *T. gondii* protein can be produced using a number of methods known to those skilled in the art, see, for example, Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press; Sambrook et al., *ibid.*, is incorporated by reference herein in its entirety. For example, nucleic acid molecules can be modified using a variety of techniques including, but not limited to, classic mutagenesis and recombinant DNA techniques such as site-directed mutagenesis, chemical treatment, restriction enzyme cleavage, ligation of nucleic acid fragments, PCR amplification, synthesis of oligonucleotide mixtures and ligation of mixture groups to "build" a mixture of nucleic acid molecules, and combinations thereof. Nucleic acid molecule homologs can be selected by hybridization with a nucleic acid molecule encoding an immunogenic *T. gondii* protein or by screening the function of a protein encoded by the nucleic acid molecule (e.g., ability to elicit an immune response against at least one epitope of an immunogenic *T. gondii* protein).

An isolated nucleic acid molecule of the present invention can include a nucleic acid sequence that encodes at least one immunogenic *T. gondii* protein of the present

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invention, examples of which are disclosed herein. Although the phrase "nucleic acid molecule" primarily refers to the physical nucleic acid molecule and the phrase "nucleic acid sequence" primarily refers to the sequence of nucleotides on the nucleic acid molecule, the two phrases can be used interchangeably, especially with respect to a nucleic acid molecule, or a nucleic acid sequence, capable of encoding an *T. gondii* protein.

A preferred nucleic acid molecule of the present invention, when administered to a cat, is capable of preventing *T. gondii* oocyst shedding. As will be disclosed in more detail below, such a nucleic acid molecule can be, or encode, an antisense RNA, a molecule capable of triple helix formation, a ribozyme, or other nucleic acid-based drug compound. In additional embodiments, a nucleic acid molecule of the present invention can encode a protective protein (e.g., an immunogenic *T. gondii* protein of the present invention), the nucleic acid molecule being delivered to the animal, for example, by direct injection (i.e, as a genetic vaccine) or in a vehicle such as a recombinant virus vaccine or a recombinant cell vaccine. Another preferred nucleic acid molecule of the present invention, when administered to an animal, is capable of preventing disease in that animal caused by *T. gondii*.

One embodiment of the present invention is an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a nucleic acid molecule comprising at least one of the nucleic acid molecules cited in Table 1. As such, also preferred is a nucleic acid molecule that hybridizes under stringent hybridization conditions with at least one of the nucleic acid sequences cited in Table 1 or with a complement of such a sequence. More preferred is a nucleic acid molecule that hybridizes under stringent hybridization conditions with at least one of the cited nucleic acid molecules. As such, also preferred is a nucleic acid molecule that hybridizes under stringent hybridization conditions with at least one of the cited nucleic acid sequences or with a complement thereof.

Even more preferred are isolated nucleic acid molecules that are at least about 75%, preferably at least about 80%, more preferably at least about 85%, even more preferably at least about 90%, even more preferably at least about 95%, and even more preferably at least about 98% identical to one of the nucleic acid molecules and/or

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nucleic acid sequences cited in Table 1. Also preferred are nucleic acid molecules that form stable hybrids with nucleic acid molecules having those percent identities.

A particularly preferred isolated nucleic acid molecule of the present invention is a nucleic acid molecule that comprises at least one of the cited nucleic acid molecules and/or cited nucleic acid sequences, a nucleic acid molecule that is an allelic variant of at least one of the cited nucleic acid molecules and/or nucleic acid sequences, or a nucleic acid molecule that is a portion thereof (i.e., a nucleic acid molecule that forms a stable hybrid with at least one of the cited nucleic acid molecules or allelic variants thereof).

In one embodiment, a nucleic acid molecule encoding an immunogenic *T. gondii* protein of the present invention encodes a protein that is at least about 75%, preferably at least about 80%, more preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to the proteins cited in Table 1. Even more preferred is a nucleic acid molecule encoding a protein cited in Table 1 or an allelic variant of such a nucleic acid molecule. Also preferred are nucleic acid molecules encoding proteins comprising one or more epitopes of proteins having the cited percent identities or epitopes of proteins cited in Table 1 or encoded by nucleic acid molecules that are allelic variants of nucleic acid molecules cited in Table 1.

In another embodiment, a nucleic acid molecule encoding an immunogenic *T. gondii* protein of the present invention encodes a protein having an amino acid sequence that is at least about 75%, preferably at least about 80%, more preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to at least one of the amino acid sequences cited in Table 1. Even more preferred is a nucleic acid molecule encoding a protein having an amino acid sequence cited in Table 1 or an allelic variant of such a nucleic acid molecule. Also preferred are nucleic acid molecules encoding proteins comprising one or more epitopes of proteins having the cited percent identities or epitopes of proteins having amino acid sequences cited in Table 1 or encoded by nucleic acid molecules that are allelic variants of nucleic acid molecules cited in Table 1.

Note that nucleic acid molecules of the present invention can include nucleotide sequences in addition to those disclosed above, such as, but not limited to, nucleotide sequences comprising a full-length gene, a full-length coding region, a nucleic acid

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molecule encoding a fusion protein, or a nucleic acid molecule encoding a multivalent protective compound. Also included in the present invention are nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed. Preferred nucleic acid molecules of the present invention include fragments of the nucleic acid molecules disclosed in Table 1.

Knowing the nucleic acid sequences of certain nucleic acid molecules encoding immunogenic T. gondii proteins of the present invention allows one skilled in the art to, for example, (a) make copies of those nucleic acid molecules, (b) obtain nucleic acid molecules including at least a portion of such nucleic acid molecules (e.g., nucleic acid molecules including full-length genes, full-length coding regions, regulatory control sequences, truncated coding regions), and (c) obtain other nucleic acid molecules encoding an immunogenic T. gondii proteins. Such nucleic acid molecules can be obtained in a variety of ways including screening appropriate expression libraries with antibodies of the present invention; traditional cloning techniques using oligonucleotide probes of the present invention to screen appropriate libraries; and PCR amplification of appropriate libraries or DNA using oligonucleotide primers of the present invention. Preferred libraries to screen or from which to amplify nucleic acid molecules include T. gondii cDNA libraries as well as genomic DNA libraries. Similarly, preferred DNA sources from which to amplify nucleic acid molecules include T. gondii cDNA and genomic DNA. Techniques to clone and amplify nucleic acid molecules are disclosed, for example, in Sambrook et al., ibid.

The present invention also includes nucleic acid molecules that are oligonucleotides capable of hybridizing, under stringent hybridization conditions, with complementary regions of other, preferably longer, nucleic acid molecules of the present invention such as those comprising nucleic acid molecules encoding immunogenic T. gondii proteins. Oligonucleotides of the present invention can be RNA, DNA, or derivatives of either. The minimum size of such oligonucleotides is the size required for formation of a stable hybrid between an oligonucleotide and a complementary sequence on a nucleic acid molecule of the present invention. A preferred oligonucleotide of the present invention has a maximum size of about 100 nucleotides. The present invention includes oligonucleotides that can be used as, for example, probes to identify nucleic

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acid molecules encoding immunogenic *T. gondii* proteins, primers to produce nucleic acid molecules encoding immunogenic *T. gondii* proteins, or reagents to inhibit immunogenic *T. gondii* protein production or activity (e.g., as antisense-, triplex formation-, ribozyme- and/or RNA drug-based reagents). The present invention also includes the use of such oligonucleotides to protect animals from disease using one or more of such technologies. Appropriate oligonucleotide-containing compositions can be administered to an animal using techniques known to those skilled in the art.

One embodiment of the present invention includes a recombinant vector, which includes at least one isolated nucleic acid molecule of the present invention, inserted into any vector capable of delivering the nucleic acid molecule into a host cell. Such a vector contains heterologous nucleic acid sequences, that is nucleic acid sequences that are not naturally found adjacent to nucleic acid molecules of the present invention and that preferably are derived from a species other than the species from which the nucleic acid molecule(s) are derived. The vector can be either RNA or DNA, either prokaryotic or eukaryotic, and typically is a virus or a plasmid. Recombinant vectors can be used in the cloning, sequencing, and/or otherwise manipulating of nucleic acid molecule encoding immunogenic *T. gondii* proteins of the present invention.

One type of recombinant vector, referred to herein as a recombinant molecule, comprises a nucleic acid molecule of the present invention operatively linked to an expression vector. The phrase operatively linked refers to insertion of a nucleic acid molecule into an expression vector in a manner such that the molecule is able to be expressed when transformed into a host cell. As used herein, an expression vector is a DNA or RNA vector that is capable of transforming a host cell and of effecting expression of a specified nucleic acid molecule. Preferably, the expression vector is also capable of replicating within the host cell. Expression vectors can be operative in either prokaryotic or eukaryotic cells, and are typically viruses or plasmids. Expression vectors of the present invention include any vectors that function (i.e., direct gene expression) in recombinant cells of the present invention, including in bacterial, fungal, parasite, insect, other animal, and plant cells. Preferred expression vectors of the present invention can direct gene expression in bacterial, yeast, *T. gondii* and mammalian cells, and more preferably in the cell types disclosed herein.

In particular, expression vectors of the present invention contain regulatory sequences such as transcription control sequences, translation control sequences, origins of replication, and other regulatory sequences that are compatible with the recombinant cell and that control the expression of nucleic acid molecules of the present invention. In particular, recombinant molecules of the present invention include transcription control sequences. Transcription control sequences are sequences which control the initiation, elongation, and termination of transcription. Particularly important transcription control sequences are those which control transcription initiation, such as promoter, enhancer, operator and repressor sequences. Suitable transcription control sequences include any transcription control sequence that can function in at least one of the recombinant cells of the present invention. A variety of such transcription control sequences are known to those skilled in the art. Preferred transcription control sequences include those which function in bacterial, yeast, helminth or other endoparasite, or insect and mammalian cells, such as, but not limited to, tac, lac, trp, trc, oxy-pro, omp/lpp, rrnB, bacteriophage lambda (such as lambda p_L and lambda p_R and fusions that include such promoters), bacteriophage T7, T7lac, bacteriophage T3, bacteriophage SP6, bacteriophage SP01, metallothionein, alpha-mating factor, Pichia alcohol oxidase, alphavirus subgenomic promoter, antibiotic resistance gene, baculovirus, Heliothis zea insect virus, vaccinia virus, herpesvirus, raccoon poxvirus, other poxvirus, adenovirus, cytomegalovirus (such as immediate early promoter), simian virus 40, retrovirus, actin, retroviral long terminal repeat, Rous sarcoma virus, heat shock, phosphate and nitrate transcription control sequences as well as other sequences capable of controlling gene expression in prokaryotic or eukaryotic cells. Additional suitable transcription control sequences include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (e.g., promoters inducible by interferons or interleukins). Transcription control sequences of the present invention can also include naturally occurring transcription control sequences naturally associated with T. gondii.

Suitable and preferred nucleic acid molecules to include in recombinant vectors of the present invention are as disclosed herein. Preferred nucleic acid molecules to include in recombinant vectors, and particularly in recombinant molecules, include those cited in Table 1. Particularly preferred recombinant molecules of the present invention

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include those recombinant molecules, the production of which are described in the Examples section.

Recombinant molecules of the present invention may also (a) contain secretory signals (i.e., signal segment nucleic acid sequences) to enable an expressed *T. gondii* protein of the present invention to be secreted from the cell that produces the pretein and/or (b) contain fusion sequences which lead to the expression of nucleic acid molecules of the present invention as fusion proteins. Examples of suitable signal segments include any signal segment capable of directing the secretion of a protein of the present invention. Preferred signal segments include, but are not limited to, tissue plasminogen activator (t-PA), interferon, interleukin, growth hormone, histocompatibility and viral envelope glycoprotein signal segments. Suitable fusion segments encoded by fusion segment nucleic acids are disclosed herein. In addition, a nucleic acid molecule of the present invention can be joined to a fusion segment that directs the encoded protein to the proteosome, such as a ubiquitin fusion segment. Eukaryotic recombinant molecules may also include intervening and/or untranslated sequences surrounding and/or within the nucleic acid sequences of nucleic acid molecules of the present invention.

Another embodiment of the present invention includes a recombinant cell comprising a host cell transformed with one or more recombinant molecules of the present invention. Transformation of a nucleic acid molecule into a cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection, lipofection, adsorption, and protoplast fusion. A recombinant cell may remain unicellular or may grow into a tissue, organ or a multicellular organism. Transformed nucleic acid molecules of the present invention can remain extrachromosomal or can integrate into one or more sites within a chromosome of the transformed (i.e., recombinant) cell in such a manner that their ability to be expressed is retained. Preferred nucleic acid molecules with which to transform a cell include nucleic acid molecules encoding immunogenic *T. gondii* proteins disclosed herein. Particularly preferred nucleic acid molecules with which to transform a cell include those listed in Table 1.

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Suitable host cells to transform include any cell that can be transformed with a nucleic acid molecule of the present invention. Host cells can be either untransformed cells or cells that are already transformed with at least one nucleic acid molecule (e.g., nucleic acid molecules encoding one or more proteins of the present invention and/or other proteins useful in the production of multivalent vaccines). Host cells of the present invention either can be endogenously (i.e., naturally) capable of producing T. gondii proteins of the present invention or can be capable of producing such proteins after being transformed with at least one nucleic acid molecule of the present invention. Host cells of the present invention can be any cell capable of producing at least one protein of the present invention, and include bacterial, fungal (including yeast), parasite (including 10 helminth, protozoa and ectoparasite), insect, other animal and plant cells. Preferred host cells include bacterial, mycobacterial, yeast, protozoan, helminth, insect and mammalian cells. More preferred host cells include Salmonella, Escherichia, Bacillus, Listeria, Saccharomyces, Spodoptera, Mycobacteria, Trichoplusia, BHK (baby hamster kidney) cells, MDCK cells (Madin-Darby canine kidney cell line), CRFK cells (Crandell feline 15 kidney cell line), CV-1 cells (African monkey kidney cell line used, for example, to culture raccoon poxvirus), COS (e.g., COS-7) cells, and Vero cells. Particularly preferred host cells are Escherichia coli, including E. coli K-12 derivatives; Salmonella typhi; Salmonella typhimurium, including attenuated strains such as UK-1 $_{\chi}$ 3987 and SR-11 $_{\chi}$ 4072; Spodoptera frugiperda; Trichoplusia ni; BHK cells; MDCK cells; CRFK 20 cells; CV-1 cells; COS cells; Vero cells; and non-tumorigenic mouse myoblast G8 cells (e.g., ATCC CRL 1246). Additional appropriate mammalian cell hosts include other kidney cell lines, other fibroblast cell lines (e.g., human, murine or chicken embryo fibroblast cell lines), myeloma cell lines, Chinese hamster ovary cells, mouse NIH/3T3 cells, LMTK³¹ cells and/or HeLa cells. In one embodiment, the proteins may be 25 expressed as heterologous proteins in myeloma cell lines employing immunoglobulin promoters.

A recombinant cell is preferably produced by transforming a host cell with one or more recombinant molecules, each comprising one or more nucleic acid molecules of the present invention operatively linked to an expression vector containing one or more transcription control sequences, examples of which are disclosed herein.

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A recombinant cell of the present invention includes any cell transformed with at least one of any nucleic acid molecule of the present invention. Suitable and preferred nucleic acid molecules as well as suitable and preferred recombinant molecules with which to transfer cells are disclosed herein. Particularly preferred recombinant cells include those recombinant cells, the production of which are disclosed in the Examples section.

Recombinant cells of the present invention can also be co-transformed with one or more recombinant molecules including a nucleic acid molecule encoding at least one immunogenic *T. gondii* protein of the present invention and one or more other nucleic acid molecules encoding other protective compounds, as disclosed herein (e.g., to produce multivalent vaccines).

Recombinant DNA technologies can be used to improve expression of transformed nucleic acid molecules by manipulating, for example, the number of copies of the nucleic acid molecules within a host cell, the efficiency with which those nucleic acid molecules are transcribed, the efficiency with which the resultant transcripts are translated, and the efficiency of post-translational modifications. Recombinant techniques useful for increasing the expression of nucleic acid molecules of the present invention include, but are not limited to, operatively linking nucleic acid molecules to high-copy number plasmids, integration of the nucleic acid molecules into one or more host cell chromosomes, addition of vector stability sequences to plasmids, substitutions or modifications of transcription control signals (e.g., promoters, operators, enhancers), substitutions or modifications of translational control signals (e.g., ribosome binding sites, Shine-Dalgarno sequences), modification of nucleic acid molecules of the present invention to correspond to the codon usage of the host cell, deletion of sequences that destabilize transcripts, and use of control signals that temporally separate recombinant cell growth from recombinant enzyme production during fermentation. The activity of an expressed recombinant protein of the present invention may be improved by fragmenting, modifying, or derivatizing nucleic acid molecules encoding such a protein.

Isolated *T. gondii* proteins of the present invention can be produced in a variety of ways, including production and recovery of natural proteins, production and recovery of recombinant proteins, and chemical synthesis of the proteins. In one embodiment, an

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isolated protein of the present invention is produced by culturing a cell capable of expressing the protein under conditions effective to produce the protein, and recovering the protein. A preferred cell to culture is a recombinant cell of the present invention. Effective culture conditions include, but are not limited to, effective media, bioreactor, temperature, pH and oxygen conditions that permit protein production. An effective medium refers to any medium in which a cell is cultured to produce an immunogenic T. gondii protein of the present invention. Effective media typically comprise an aqueous medium having assimilable carbon, nitrogen and phosphate sources, and appropriate salts, minerals, metals and other nutrients, such as vitamins. Cells of the present invention can be cultured in conventional fermentation bioreactors, shake flasks, test tubes, microtiter dishes, and petri plates. Culturing can be carried out at a temperature, pH and oxygen content appropriate for a recombinant cell. Suitable culturing conditions are within the expertise of one of ordinary skill in the art. Examples of suitable conditions are included in the Examples section.

Depending on the vector and host system used for production, resultant proteins of the present invention may either remain within the recombinant cell; be secreted into the fermentation medium; be secreted into a space between two cellular membranes, such as the periplasmic space in *E. coli*; or be retained on the outer surface of a cell or viral membrane.

The phrase "recovering the protein", as well as similar phrases, refers to collecting the whole fermentation medium containing the protein and need not imply additional steps of separation or purification. Proteins of the present invention can be purified using a variety of standard protein purification techniques, such as, but not limited to, affinity chromatography, ion exchange chromatography, filtration, electrophoresis, hydrophobic interaction chromatography, gel filtration chromatography, reverse phase chromatography, concanavalin A chromatography, chromatofocusing and differential solubilization. Proteins of the present invention are preferably retrieved in "substantially pure" form. As used herein, "substantially pure" refers to a purity that allows for the effective use of the protein as a composition to inhibit *T. gondii* oocyst shedding in a cat due to infection with *T. gondii*, or for preventing *T. gondii* infection in an animal, or as a diagnostic reagent. A composition for inhibiting *T. gondii* oocyst

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shedding in a cat due to infection with *T. gondii* animals, or for preventing *T. gondii* infection in an animal for example, should exhibit no substantial toxicity and preferably should be capable of stimulating the production of antibodies in a treated animal.

The present invention also includes isolated (i.c., removed from their natural milieu) antibodies that selectively bind to an immunogenic *T. gondii* protein of the present invention or a mimetope thereof (e.g., anti-*T. gondii* antibodies). As used herein, the term "selectively binds to" an immunogenic *T. gondii* protein refers to the ability of antibodies of the present invention to preferentially bind to specified proteins and mimetopes thereof of the present invention. Binding can be measured using a variety of methods standard in the art including enzyme immunoassays (e.g., ELISA), immunoblot assays, etc.; see, for example, Sambrook et al., *ibid.*, and Harlow, et al., 1988, *Antibodies, a Laboratory Manual*, Cold Spring Harbor Labs Press; Harlow et al., *ibid.*, is incorporated by reference herein in its entirety. An anti-*T. gondii* antibody of the present invention preferably selectively binds to an immunogenic *T. gondii* protein in such a way as to inhibit the function of that protein.

Isolated antibodies of the present invention can include antibodies in any bodily fluid that has been collected (e.g., recovered) from an animal. Suitable bodily fluids include, but are not limited to, blood, serum, plasma, urine, tears, aqueous humor, central nervous system fluid (CNF), saliva, lymph, nasal secretions, milk and feces. Thus, serum containing antibodies (i.e., antiserum) or mucosal secretions, such as intestinal secretions, are examples of isolated antibodies. Other embodiments of antibodies include antibodies that have been purified to varying degrees. Antibodies of the present invention can be polyclonal or monoclonal, or can be functional equivalents such as antibody fragments and genetically-engineered antibodies, including single chain antibodies or chimeric antibodies that can bind to one or more epitopes.

A preferred method to produce antibodies of the present invention includes (a) administering to an animal an effective amount of a protein, peptide or mimetope thereof of the present invention to produce the antibodies and (b) recovering the antibodies. In another method, antibodies of the present invention are produced recombinantly using techniques as heretofore disclosed to produce *T. gondii* proteins of the present invention.

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Antibodies raised against defined proteins or mimetopes can be advantageous because such antibodies are not substantially contaminated with antibodies against other substances that might otherwise cause interference in a diagnostic assay or side effects if used in a composition for inhibiting *T. gondii* oocyst shedding in a cat due to infection with *T. gondii*, or for preventing *T. gondii* infection in an animal.

Antibodies of the present invention have a variety of potential uses that are within the scope of the present invention. For example, such antibodies can be used (a) as compounds to passively immunize a cat in order to inhibit the cat from shedding T. gondii oocysts, (b) as reagents in assays to detect infection by T. gondii and/or (c) as tools to screen expression libraries and/or to recover desired proteins of the present invention from a mixture of proteins and other contaminants.

One embodiment of the present invention includes a method for identifying a nucleic acid molecule encoding an immunogenic T. gondii protein. According to this method, antiserum (comprising either monoclonal or polyclonal antibodies) raised against a T. gondii developmental stage or stages, or against oocysts, is used to immunoscreen a T. gondii genomic expression library or a T. gondii cDNA expression library, and a nucleic acid molecule expressing an immunogenic T. gondii protein is identified by its ability to selectively bind to at least one antibody within the antiserum. As used herein, the term immunoscreen refers to a method in which antibodies are mixed with a sample to determine whether the sample contains a substance to which the antibodies can selectively bind. A substance is identified by its ability to selectively bind to such antibodies. Although general methods to accomplishing immunoscreening of expression libraries are known to those skilled in the art, the exact method to use such a technique to identify T. gondii immunogenic proteins was not previously known. The present invention includes the identification of antisera that are useful in the identification and isolation of nucleic acid molecules encoding T. gondii immunogenic proteins. Such antisera include antiserum raised against T. gondii oocysts, antiserum raised against T. gondii bradyzoites, antiserum raised against T. gondii infected cat gut, and antiserum isolated from a cat immune to T. gondii infection. In one embodiment, antiserum as described above is enriched for antibodies specific to T. gondii

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gametogenic stages. In a preferred embodiment, polyclonal antiserum is produced by exposing an animal to a *T. gondii* antigen or antigens, then isolating the antiserum from the animal so exposed. Methods to produce and use the various antisera are described in the Examples section.

In another embodiment, immunoscreening as described above can be used to identify an immunogenic *T. gondii* protein. According to this method, antiserum as described above is used to immunoscreen a *T. gondii* genomic expression library or cDNA expression library, and an immunogenic T. gondii protein is identified. *T. gondii* immunogenic proteins can also be identified by immunoscreening preparations containing *T. gondii* antigens (e.g., *T. gondii* oocysts, bradyzoites, infected cat guts) using antiserum as described above.

Nucleic acid molecules and proteins identified using such techniques can be isolated (i.e., recovered) and purified to a desired state of purity using techniques known to those skilled in the art.

One embodiment of the present invention is a composition that, when administered to a cat in an effective manner, is capable of preventing that cat from shedding T. gondii oocysts. Compositions of the present invention, useful for inhibiting T. gondii oocyst shedding in a cat due to infection with T. gondii (i.e., infection with T. gondii causes oocyst shedding in cats), include at least one of the following protective compounds: an isolated immunogenic T. gondii protein or a mimetope thereof, an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a nucleic acid molecule comprising one of the nucleic acid molecules and/or nucleic acid sequences cited in Table 1, an isolated antibody that selectively binds to an immunogenic T. gondii protein, an inhibitor of T. gondii function identified by its ability to bind to an immunogenic T. gondii protein and thereby impede development and/or the production of oocysts, or a mixture thereof (i.e., combination of at least two of the compounds). As used herein, a protective compound refers to a compound that, when administered to a cat in an effective manner, is able to inhibit the cat from shedding T. gondii oocysts upon infection with T. gondii. The term protective compound also refers to a compound that, when administered to a cat or other animal, including a human, in

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an effective manner, is able to prevent or ameliorate disease caused by infection with T. gondii. Examples of proteins, nucleic acid molecules, antibodies and inhibitors of the present invention are disclosed herein.

The present invention also includes a composition comprising at least one T. gondii protein-based compound of the present invention in combination with at least one additional compound protective against one or more infectious agents. Examples of such compounds and infectious agents are disclosed herein.

Compositions of the present invention that are useful for preventing T. gondii infection can be administered to any animal susceptible to such therapy, preferably to mammals.

In order to inhibit a cat from shedding T. gondii oocysts, a composition of the present invention is administered to the cat in a manner effective to inhibit that cat from shedding T. gondii oocysts. In a preferred embodiment, compositions of the present invention are administered to cats prior to infection in order to prevent oocyst shedding (i.e., as a preventative vaccine). In another embodiment, compositions of the present invention can be administered to animals after infection in order to treat disease caused by T. gondii (e.g., as a therapeutic vaccine).

Compositions of the present invention, useful for inhibiting T. gondii oocyst shedding in a cat due to infection with T. gondii, or for preventing T. gondii infection in an animal, can be formulated in an excipient that the animal to be treated can tolerate. Examples of such excipients include water, saline, Ringer's solution, dextrose solution, Hank's solution, and other aqueous physiologically balanced salt solutions. Nonaqueous vehicles, such as fixed oils, sesame oil, ethyl oleate, or triglycerides may also be used. Other useful formulations include suspensions containing viscosity enhancing agents, such as sodium carboxymethylcellulose, sorbitol, or dextran. Excipients can also contain 25 minor amounts of additives, such as substances that enhance isotonicity and chemical stability. Examples of buffers include phosphate buffer, bicarbonate buffer and Tris buffer, while examples of preservatives include thimerosal, — or o-cresol, formalin and benzyl alcohol. Standard formulations can either be liquid injectables or solids which can be taken up in a suitable liquid as a suspension or solution for injection. Thus, in a 30

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non-liquid formulation, the excipient can comprise dextrose, human serum albumin, preservatives, etc., to which sterile water or saline can be added prior to administration.

In one embodiment of the present invention, a composition useful for inhibiting oocyst shedding in a cat infected with T. gondii, or for preventing T. gondii infection in an animal, can include an adjuvant. Adjuvants are agents that are capable of enhancing the immune response of an animal to a specific antigen. Suitable adjuvants include, but are not limited to, cytokines, chemokines, and compounds that induce the production of cytokines and chemokines (e.g., granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), colony stimulating factor (CSF), erythropoietin (EPO), interleukin 2 (IL-2), interleukin-3 (IL-3), interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 6 (IL-6), interleukin 7 (IL-7), interleukin 8 (IL-8), interleukin 10 (IL-10), interleukin 12 (IL-12), interferon gamma, interferon gamma inducing factor I (IGIF), transforming growth factor beta, RANTES (regulated upon activation, normal T-cell expressed and presumably secreted), macrophage inflammatory proteins (e.g., MIP-1 alpha and MIP-1 beta), and Leishmania elongation initiating factor (LEIF)); bacterial components (e.g., endotoxins, in particular superantigens, exotoxins and cell wall components); aluminumbased salts; calcium-based salts; silica; polynucleotides; toxoids; serum proteins, viral coat proteins; block copolymer adjuvants (e.g., Hunter's Titermax™ adjuvant (Vaxcel™, Inc. Norcross, GA), Ribi adjuvants (Ribi ImmunoChem Research, Inc., Hamilton, MT); and saponins and their derivatives (e.g., Quil A (Superfos Biosector A/S, Denmark). Protein adjuvants of the present invention can be delivered in the form of the protein themselves or of nucleic acid molecules encoding such proteins using the methods described herein.

In one embodiment of the present invention, a composition useful for inhibiting oocyst shedding in a cat infected with T. gondii, or for preventing T. gondii infection in an animal, can include a carrier. Carriers include compounds that increase the half-life of a composition of the present invention in the treated animal. Suitable carriers include, but are not limited to, polymeric controlled release vehicles, biodegradable implants, liposomes, bacteria, viruses, other cells, oils, esters, and glycols.

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One embodiment of the present invention is a controlled release formulation that is capable of slowly releasing a composition of the present invention into an animal. As used herein, a controlled release formulation comprises a composition of the present invention in a controlled release vehicle. Suitable controlled release vehicles include, but are not limited to, biocompatible polymers, other polymeric matrices, capsules, microcapsules, microparticles, bolus preparations, osmotic pumps, diffusion devices, liposomes, lipospheres, and transdermal delivery systems. Other controlled release formulations of the present invention include liquids that, upon administration to an animal, form a solid or a gel *in situ*. Preferred controlled release formulations are biodegradable (i.e., bioerodible).

A preferred controlled release formulation of the present invention is capable of releasing a composition of the present invention into the blood of the treated animal at a constant rate sufficient to attain dose levels of the composition effective to either inhibit oocyst shedding by cats, or to protect an animal from disease caused by *T. gondii*. The composition is preferably released over a period of time ranging from about 1 to about 12 months. A controlled release formulation of the present invention is capable of effecting a treatment preferably for at least about 1 month, more preferably for at least about 3 months, even more preferably for at least about 6 months, even more preferably for at least about 12 months.

Compositions of the present invention can be administered to cats prior to infection in order to inhibit oocyst shedding, and/or can be administered to cats or other animals, including humans, before infection in order to prevent disease caused by T. gondii infection, or after infection in order to treat disease caused by T. gondii. For example, rucleic acid molecules, proteins, mimetopes thereof, antibodies thereof, and inhibitors thereof can be used to treat or prevent disease caused by T. gondii infection. Acceptable protocols to administer compositions of the present invention include individual dose size, number of doses, frequency of dose administration, and mode of administration. Determination of such protocols can be accomplished by those skilled in the art. A suitable single dose is a dose that is capable of protecting an animal from disease when administered one or more times over a suitable time period. For example, a preferred single dose of a protein, mimetope or antibody composition of the present

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invention is from about 1 microgram (µg) to about 10 milligrams (mg) of the composition per kilogram body weight of the animal. Booster doses can be administered from about 2 weeks to several years after the original administration. Booster administrations preferably are administered when the immune response of the animal becomes insufficient to protect the animal from disease. A preferred administration schedule is one in which from about 10 µg to about 1 mg of the composition per kg body weight of the animal is administered from about one to about two times over a time period of from about 2 weeks to about 12 months. Modes of administration can include, but are not limited to, injection, oral administration, inhalation, nasal administration, intraocular administration, anal administration, topical administration, particle bombardment, and intradermal scarification. Preferred injection methods include intradermal, intramuscular, subcutaneous, intravenous methods, with intradermal injection and intramuscular injection being more preferred. A particularly preferred method is mucosal administration.

According to one embodiment, a nucleic acid molecule of the present invention can be administered to an animal in a fashion to enable expression of that nucleic acid molecule into a protective protein or protective RNA (e.g., antisense RNA, ribozyme, triple helix forms or RNA drug) in the animal. Nucleic acid molecules can be delivered to an animal in a variety of methods including, but not limited to, (a) administering a nucleic acid not packaged in a viral coat or cell as a genetic vaccine (e.g., as "naked" DNA or RNA molecules with or without a non-viral/non-cellular carrier (e.g., liposome, hydrogel, etc.) or (b) administering a nucleic acid molecule packaged as a recombinant virus vaccine or as a recombinant cell vaccine (i.e., the nucleic acid molecule is delivered by a viral or cellular vehicle).

A genetic vaccine of the present invention includes a recombinant molecule of the present invention. As such, a genetic vaccine comprises at least one isolated nucleic acid molecule encoding an immunogenic *T. gondii* protein operatively linked to a eukaryotic or prokaryotic transcription control region. A genetic vaccine can be either RNA or DNA, can have components from prokaryotic as well as eukaryotic sources, and can have the ability, by methods described herein, to enter either eukaryotic or prokaryotic cells and direct expression of isolated nucleic acid molecules of the present

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invention in those cells. In a preferred embodiment, a genetic vaccine of the present invention includes a recombinant virus genome (i.e., a nucleic acid molecule of the present invention ligated to at least one viral genome in which transcription of the nucleic acid molecule is directed either by a transcription control region on the genome or a separate transcriptio:, control region) or a recombinant plasmid that includes a nucleic acid molecule of the present invention ligated into a vector that is not a viral genome such that the nucleic acid molecule is operatively linked to a transcription control region.

A genetic vaccine of the present invention includes a nucleic acid molecule of the present invention and preferably includes a recombinant molecule of the present invention that preferably is replication, or otherwise amplification, competent. A genetic vaccine of the present invention can comprise one or more nucleic acid molecules of the present invention in the form of, for example, a dicistronic recombinant molecule. Preferred genetic vaccines include at least a portion of a viral genome (i.e., a viral vector) and a nucleic acid molecule of the present invention. Preferred viral vectors include those based on alphaviruses, poxviruses, adenoviruses, adeno-associated viruses, herpesviruses, picornaviruses, and retroviruses, with those based on alphaviruses (e.g., Sindbis virus or Semliki forest virus), picornaviruses (e.g., poliovirus or mengovirus), species-specific herpesviruses and poxviruses being particularly preferred. Any suitable transcription control sequence can be used, including those disclosed as suitable for protein production. Particularly preferred transcription control sequences include cytomegalovirus immediate early (preferably in conjunction with Intron-A), Rous sarcoma virus long terminal repeat, and tissue-specific transcription control sequences, as well as transcription control sequences endogenous to viral vectors if viral vectors are used. The incorporation of a "strong" polyadenylation signal is also preferred. 25

Genetic vaccines of the present invention can be administered in a variety of ways, with intramuscular, subcutaneous, intradermal, transdermal, intraocular, intranasal and oral routes of administration being preferred. A preferred single dose of a genetic vaccine ranges from about 1 nanogram (ng) to about 600 µg, depending on the route of administration and/or method of delivery, as can be determined by those skilled in the art. Suitable delivery methods include, for example, by injection, by gene gun, as drops,

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as inhaled aerosols, ingested in microparticles or microcapsules, and/or topical delivery. Genetic vaccines of the present invention can be contained in an aqueous excipient (e.g., phosphate buffered saline) alone or in a carrier (e.g., lipid-based vehicles).

A recombinant virus vaccine of the present invention includes a recombinant molecule of the present invention that is packaged in a viral coat and that can be expressed in an animal after administration. Preferably, the recombinant molecule is packaging- or replication-deficient and/or encodes an attenuated virus. A number of recombinant viruses can be used, including, but not limited to, those based on alphaviruses, poxviruses, adenoviruses, herpesviruses, picornaviruses, and retroviruses. Preferred recombinant virus vaccines are those based on alphaviruses (e.g., Sindbis virus), picornaviruses (e.g., poliovirus, mengovirus), raccoon poxviruses, speciesspecific herpesviruses and species-specific poxviruses. An example of methods to produce and use alphavirus recombinant virus vaccines are disclosed in PCT Publication No. WO 94/17813, by Xiong et al., published August 18, 1994, which is incorporated by reference herein in its entirety.

When administered to an animal, a recombinant virus vaccine of the present invention infects cells within the immunized animal and directs the production of a protective protein or RNA nucleic acid molecule that is capable of preventing a cat from shedding oocysts as disclosed herein. For example, a recombinant virus vaccine comprising a nucleic acid molecule encoding an immunogenic *T. gondii* protein of the present invention is administered according to a protocol that results in the subject cat producing a sufficient immune response to inhibit shedding *T. gondii* oocysts. A preferred single dose of a recombinant virus vaccine of the present invention is from about 1 x 10⁴ to about 1 x 10⁸ virus plaque forming units (pfu) per kilogram body weight of the animal. Administration protocols are similar to those described herein for protein-based vaccines, with subcutaneous, intramuscular, intraocular, intranasal and oral administration routes being preferred.

A recombinant cell vaccine of the present invention includes recombinant cells of the present invention that express at least one protein of the present invention. Preferred recombinant cells for this embodiment include Salmonella, E. coli, Listeria, Mycobacterium, S. frugiperda, yeast, (including Saccharomyces cerevisiae and Pichia

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pastoris), BHK, CV-1, myoblast G8, COS (e.g., COS-7), Vero, MDCK and CRFK recombinant cells. Recombinant cell vaccines of the present invention can be administered in a variety of ways but have the advantage that they can be administered orally, preferably at doses ranging from about 108 to about 1012 cells per kilogram body weight. Administration protocols are similar to those described herein for protein-based vaccines. Recombinant cell vaccines can comprise whole cells, cells stripped of cell walls or cell lysates.

The efficacy of a composition of the present invention to inhibit oocyst shedding caused by T. gondii can be tested in a variety of ways including, but not limited to, detection of protective antibodies (using, for example, proteins or mimetopes of the present invention), detection of cellular immunity within the treated animal, or challenge of the treated animal with T. gondii to determine whether the treated animal is resistant to oocyst shedding. Challenge studies can include direct administration of T. gondii tachyzoites or tissue cysts or sporulated oocysts (the infective stages) to the treated animal. In one embodiment, compositions of the present invention can be tested in animal models such as mice. Such techniques are known to those skilled in the art.

One preferred embodiment of the present invention is the use of immunogenic T. gondii proteins, nucleic acid molecules encoding immunogenic T. gondii proteins, antibodies and inhibitors of the present invention, to inhibit a cat from shedding oocysts. It is particularly preferred to prevent intestinal stages of the parasite from developing into oocysts. Preferred compositions are those that are able to inhibit at least one step in the portion of the parasite's development cycle that occurs in the intestines prior to the development of oocysts. In cats infected with tissue cysts, for example, the prepatent period for oocyst shedding is three to five days. When cats are infected with sporulated oocysts, for example, the prepatent period can range from 19 to 45 days. Particularly preferred compositions useful for inhibiting oocyst shedding in a cat infected with T. gondii include T. gondii-based compositions of the present invention. Such compositions include nucleic acid molecules encoding immunogenic T. gondii proteins, immunogenic T. gondii proteins and mimetopes thereof and anti-T. gondii antibodies. Compositions of the present invention are administered to cats in a manner effective to inhibit the cats from shedding T. gondii oocysts. Additional protection may be obtained 30

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by administering additional protective compounds, including other *T. gondii* proteins, nucleic acid molecules and antibodies, as disclosed herein.

It is also within the scope of the present invention to use isolated proteins, mimetopes, nucleic acid molecules and antibodies of the present invention as diagnostic reagents to detect infection by *T. gondii*. These diagnostic reagents can further be supplemented with additional compounds that can specifically detect any or all phases of the parasite's life cycle. General methods to use diagnostic reagents in the diagnosis of disease are known to those skilled in the art. A method or a kit for the detection of *T. gondii* infection could be combined with reagents for the detection of additional infectious agents, for example viruses (e.g. Coronaviruses), bacteria (e.g. *Campylobacter, Clostridium, Salmonella*), protozoa (e.g. *Cryptosporidium, Giardia, Isospora, Hammondia, Sarcocystis, Besnoitia, Microsporidium*), and/or multi-cellular organisms (e.g. *Teania, Anclostoma, Toxocara, Physaloptera, Paragonimus, Strongyloides, Trichuris*).

Another embodiment of the present invention is a method to detect microscopic parasite cysts or oocysts in feces using PCR amplification techniques. By microscopic, it is meant cysts or oocysts that are too small to be conveniently detected by simple visual observation of the feces. Preferred organisms to be detected include oocysts from infectious protozoan parasites including members of the apicomplexa and others including, for example, Toxoplasma, Cryptosporidium, Isospora, Giardia, Eimeria, Hammondia, Sarcocystis, Besnoitia, Microsporidium. Additional infectious agents to detect include, for example, viruses (e.g. Coronaviruses), bacteria (e.g. Campylobacter, Clostridium, Salmonella), and/or multi-cellular organisms (e.g. Teania, Anclostoma. Toxocara, Physaloptera, Paragonimus, Strongyloides, Trichuris). Particularly preferred oocysts to be detected include Toxoplasma and Cryptosporidium oocysts. Preferred cysts to be detected include any cysts capable of binding to a solid support and remaining bound to the support through a washing step. Preferred cysts include Giardia cysts. According to this embodiment of the invention, a solid support that is capable of binding cysts or oocysts is contacted with a sample of feces, which may or may not have been partially solubilized first in an aqueous solution, and the sample of feces is allowed 5

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to dry on the support. The solid support can be of any material to which the cysts or oocyts will bind and remain bound during washing in an aqueous solution. The support can comprise one or more compounds that aid in PCR amplification of the sample, for example by allowing the inhibitors to be released in the wash step, or by binding inhibitors of PCR that are not released in the elution step, or by otherwise inactivating inhibitors of PCR amplification. Preferred supports comprise a paper substrate to which the oocysts or cysts can bind. Preferred supports include IsoCodeJTM Stix, or their equivalent, S&S® #903™, or their equivalent, or Nobuto Blood Filter Strips, or their equivalent. The support, or the portion of the support contacted with the sample of feces, is preferably small enough to fit into a container convenient for the wash step; eg., a size that will fit into a 1.5. ml conical centrifuge tube. The portion of the support that is contacted with the sample of feces can be removed from the rest of the support in order to achieve a convenient size. The portion of the support that includes the dried sample of feces is then washed with an aqueous solution. In a preferred embodiment the aqueous solution is water, preferably distilled water. The solution can comprise one or more compounds that aid in PCR amplification of the sample, for example by inactivating or removing inhibitors of PCR amplification. DNA associated with the sample is eluted by adding an aqueous solution to the support and then heating the solution to a temperature sufficient to elute DNA from the sample, into the solution. In a preferred embodiment, the aqueous solution into which the sample is cluted is water, preferably distilled water. This solution can comprise one or more compounds that aid in PCR amplification of the sample, for example by inactivating inhibitors of PCR amplification, or by improving reaction conditions for the PCR reaction. The heating step comprises heating to a temperature sufficient to elute DNA from the sample. A preferred temperature is approximately 95° C. Oocyst or cyst-specific DNA in the elution solution is then PCR amplified using primers specific to the oocysts or cysts being detected. The amplification products indicative of oocysts or cysts are then detected using any means available for the detection of PCR amplification products. These can include, for example, separation and observation of the PCR products on a gel, or detection and/or quantification by PCR ELISA. In a preferred embodiment of

the present invention, nucleic acid molecules of the present invention are used for the detection of *T. gondii* oocysts in cat feces by PCR amplification using nucleic acid molecules of the present invention as primers. According to the present invention, detection of oocysts can be accomplished by direct analysis of feces. Methods to conduct such an assay are described further in the Examples section.

The following examples are provided for the purposes of illustration and are not intended to limit the scope of the present invention.

EXAMPLES

It is to be noted that the examples include a number of molecular biology, microbiology, immunology and biochemistry techniques considered to be familiar to those skilled in the art. Disclosure of such techniques can be found, for example, in Sambrook et al., *ibid.* and Ausubel, et al., 1993, *Current Protocols in Molecular Biology*, Greene/Wiley Interscience, New York, NY, and related references. Ausubel, et al, *ibid.* is incorporated by reference herein in its entirety. DNA sequence analysis and protein translations were carried out using the DNAsis program (available from Hitachi Software, San Bruno, CA) or MacVector program (available from International Biotechnologies, Inc., Hew Haven, CT). It should also be noted that since nucleic acid sequencing technology, and in particular the sequencing of PCR products, is not entirely error-free, that the nucleic acid sequences presented herein represent apparent nucleic acid sequences of the nucleic acid molecules encoding immunogenic *T. gondii* proteins of the present invention.

Example 1:

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This example discloses the construction of a *T. gondii* genomic expression library.

Pure mRNA from T. gondii parasite present in the infected cat gut cannot presently be obtained. Therefore, a true cDNA library for the gametogenic stages cannot be produced. In order to get around the unavailability of pure mRNA from gut stages of T. gondii, a genomic expression library in λ gt11 was constructed using Toxoplasma genomic DNA obtained from tachyzoites produced in tissue culture. This library represented genes expressed at all stages of the Toxoplasma life cycle, including the gametogenic genes.

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Construction of the library was modeled on procedures used previously for standard lambda cloning (see, for example, Sambrook, et al., <u>ibid.</u>). In brief, a series of high frequency cutting restriction enzymes were used to generate near random fragments of DNA representing the tachyzoite genome. DNA fragments of approximately 500 to 2000 bp were size selected and then inserted in frame with the expressed fusion protein in λ gtl1. Construction of this library is described in greater detail below.

Standard Production of Tachyzoites from liquid nitrogen stocks: Liquid nitrogen stocks of Toxoplasma tachyzoites (TZ) (1 ml samples at 2-4 X 10⁶ TZ/ml) were thawed in a 37°C waterbath. The samples were thawed completely without attaining 37°C.

Room temperature TMM (DMEM + 3% FBS + 0.1 ml 50 mg/ml gentamicin per 100 ml media) was added to the thawed sample according to the following timetable: 0.3 ml added at 0 minutes; 0.6 ml added at 5 minutes; 1.5 ml added at 10 minutes. The samples were maintained at room temperature for 5 minutes longer, then centrifuged for 10 minutes at 2,000 RPM at room temperature. The supernatant was discarded and the pellet resuspended in 12 ml of TMM.

Human foreskin fibroblasts (HSF)cells (ATCC CRL 1637) were infected with the thawed tachyzoites as follows: Passage 15-25 HSF cells were split 1:3 and grown to confluence in a T75 flask with DMEM + 10% FBS (fetal bovine serum, available from Summit Biotechnology, Fort Collins, CO) + 0.1 ml gentamicin per 100 ml media in an incubator at 37°C with 5% CO₂. HSF cells were infected by replacing the media with the thawed tachyzoites in TMM. Infections were allowed to progress until 30-50% of the cell monolayer was destroyed. The medium in the infected T75 flask was replaced with fresh TMM the day before harvesting tachyzoites for expansion of the culture.

Passage 19-25 HSF cells cultured in roller bottles (850 cm²), were split 1:3 and grown to confluence in a roller bottle incubator apparatus under conditions as described above. The medium from a single roller bottle was decanted and replaced with 100 ml of TMM. The cells in this roller bottle were then infected by adding medium from an infected T75 flask (described above). Infection was allowed to progress until 30-50% of the cell monolayer was destroyed. Fresh TMM was replaced in the infected roller bottle the day before using the supernatant to infect new HSF cells. Four new roller bottles with confluent HSF cells were each infected with 2.5 X 107 tachyzoites harvested from a

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previously infected roller bottle. This cycle of infection of four roller bottles, for the purpose of tachyzoite production, was continued on a weekly basis.

Tachyzoite Purification: Extracellular tachyzoites were collected from tissue culture and concentrated. To collect and concentrate tachyzoites, media from roller bottles containing extracellular tachyzoites were poured into 50 ml conical tubes and centrifuged at 2,000 RPM for 10 minutes. The resulting pellets were pooled and the volume was brought up to 50 ml using TMM. The tachyzoites were diluted and counted using a haemacytometer, and then purified by either the CF-11 column method or the nucleopore method as follows:

CF-11 Method of Purifying Tachyzoites: 1.5g of CF-11 (available from Whatman, Inc., Clifton, NJ) was mixed throughly in 50 ml of DMEM (no FBS), then added to an econo-column chromatography column (available from Biorad, Hercules, CA) and allowed to settle, forming a flat bed. The stopcock was then opened and the excess DMEM was drained until ¼ inch of media remained above the bed. The column was washed by gently adding 50 ml of DMEM and then bringing the media level down to 1 inch above the CF-11 bed. The 50 ml of tachyzoites in TMM (prepared as described above) was then added to the column. The stopcock was opened and the tachyzoites were eluted at a rate of 1 drop/second and collected into 50 ml conical tubes on ice. The media was eluted to ¼ inch above the gel bed. Two additional 5 ml elutions were performed, followed by a 40 ml elution. The 100 ml total eluate was then centrifuged at 2,000 RPM for 10 minutes. The pellets were again pooled by resuspension in 50 ml of DMEM. The tachyzoites were counted and the final number of organisms determined. The tachyzoites were centrifuged at 2,000 rpm for 10 minutes, and the pellet resuspended in 1 ml of Hanks Balanced Salt Solution (HBSS). The tachyzoites were washed 3 times with 1 ml of HBSS by centrifugation at 5000 rpm for 5 minutes in an Eppendorf centrifuge. The pellets were stored at -70°C until needed.

Nucleopore Method of Purifying Tachyzoites: 47 mm nucleopore units (available from Corning Costar Corp., Cambridge, MA) with a polycarbonate 3 um capillary pore membrane were assembled according to manufacturer's specifications. The nucleopore units were then placed on top of an open 50 ml conical tube. Five ml of DMEM was gently forced through the unit using a 30 cc syringe that connects to the top

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of the nucleopore unit. Twenty-five ml of the extracellular tachyzoite preparation collected from tissue culture in DMEM were passed through the unit by gently pushing on the 30 cc syringe. The maximum number of tachyzoites per nucleopore filter did not exceed 5 x 108. Filtration was followed by 2, 5 ml washes of DMEM. The nucleoporepurified tachyzoites were then centrifuged at 2,000 RPM for 10 minutes, and the pelleted tachyzoites resuspended in 50 ml of DMEM. The number of tachyzoites was determined by counting in a hemacytometer. Following centrifugation at 2,000 rpm for 10 minutes, the pellet was resuspended in 1 ml HBSS. The tachyzoites were washed 3 times with 1 ml of HBSS by centrifugation at 5,000 rpm for 5 minutes in an Eppendorf centrifuge. The pellets were stored at -70°C until needed.

Isolation of tachyzoite DNA: DNA from all sources (for example, DNA from Toxoplasma or mammalian tissue) was isolated using standard techniques that can be can be found, for example, in Sambrook et al, ibid. In particular, 2X109 tachyzoites were resuspended in 10 ml of 10 mM Tris, pH 8, 0.1 M EDTA, 0.5% SDS and 20 $\mu g/ml$ pancreatic RNase (available from Sigma Chemical Co., St. Louis, MO). After incubating for 1 hour at 37°C, 1 ml of 5M NaCl and 100 µl of 10 mg/ml proteinase K (available from Boehringer Mannheim Corp., Indianapolis, IN) was added and the solution incubated for 3 hours at 50°C. The solution was then extracted with phenol and the DNA precipitated with EtOH.

Preparation of Restricted and Size Selected DNA: Six, four-base recognition site restriction enzymes, Alu I, Mbo I, Msp I, Rsa I, Sau3A I, and Taq I, (available from New England Biolabs, Beverly, MA) and one six-nucleotide recognition site restriction enzyme, Dra I, were used to cut T. gondii genomic DNA to completion. Ten µg of tachyzoite DNA was digested to completion according to the manufacturer's recommended protocols for each enzyme. All seven digests of DNA were combined and electrophoresed on an 0.8% preparative agarose gel. The region of the gel representing double stranded DNA between 500 and 2000 bp was excised and the DNA recovered using a Gene Clean Kit (available from BIO 101 Inc., Vista, CA). The eluted DNA was quantitated using an ethidium bromide sensitivity assay on agarose, using calf thymus

DNA as a standard. The DNA was then ethanol precipitated. 30

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Addition of Linkers: Four µg of the digested and size selected DNA was then prepared for the addition of linkers by filling in the restriction site overhangs as follows: First, the DNA was resuspended into Klenow buffer, 0.2mM dNTPs, and Klenow fragment (available from Boehringer Mannheim Biochemicals, Indianapolis, IN), and the 5 reaction mix was incubated for 30 minutes at room temperature. The reaction was stopped by incubating the reaction mix at 65°C for 10 minutes. The DNA was then methylated using standard conditions including 0.1 mM s-adenosylmethionine and 120 units of EcoR I methylase (available from Promega Corp., Madison, WI). Following reprecipitation with ethanol, the DNA pellet was dissolved in water and standard T4 10 DNA ligase buffer (see, for example, Sambrook, et al., ibid.). Three separate EcoR I linkers, constructed to allow three different reading frames (available from Stratagene, La Jolla, CA) were added along with T4 DNA ligase (available from Promega, Corp.) and incubated for 16 hours at 15°C. The solution was then diluted directly into EcoR I restriction buffer and EcoR I enzyme (available from Promega Corp.) and incubated at 15 37°C for 2 hours. The DNA fragments were separated from the free linkers using a Sephacryl S-400 spin column. The recovered DNA was ethanol precipitated.

Ligation and Packaging of the Restricted DNA: The entire fraction of DNA obtained from the above reaction mixture was ligated into 1 μ g of EcoR I-cut and phosphatase treated λ gt11 arms (available from Stratagene) with T4 DNA ligase at 15⁰ C for 16 hours. The phage was then packaged, titered and amplified using the Gigapack® II Packaging system (available from Stratagene) according to the manufacturer's directions. The resulting library is referred to herein as the Toxoplasma or T. gondii genomic expression library or as the λ gt11:Toxoplasma genomic expression library.

25 <u>Example 2</u>:

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This Example discloses a method of isolation of *T. gondii* nucleic acid molecules encoding immunogenic *T. gondii* proteins recognized by antisera specific for a Toxoplasma intestinal stage: oocysts. This Example further discloses recombinant nucleic acid molecules, proteins and cells of the present invention.

The final stage of *T. gondii* gametogony is the unsporulated oocyst. Antisera was raised directly against Toxoplasma oocysts. In addition to the antisera reacting with their

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respective immunogens, the ability of this antisera to react with T. gondii gametogenic stages in intestinal tissue sections from infected animals was assessed. When used in immunofluorescence assays conducted on infected cat gut samples, the anti-oocyst antisera reacted with various parasite structures in the ICG tissue sections, indicating some cross-reactivity with gametogenic stages. This antisera was made as follows.

Production of antibody to a Toxoplasma intestinal stage: oocysts: Oocysts from a wild type strain designated Maggie, a recent isolate from a cat with Toxoplasmosis (Veterinary Teaching Hospital, Colorado State University, 1993), were obtained from the feces of cats fed mouse brains from mice previously infected with the Maggie strain. The oocysts were purified by the standard method of repeated sugar flotation (described in Dubey, J.P. and Beattie, C.P., (1988) Toxoplasmosis of Animals and Man, CRC Press, Boca Raton, FL). The oocysts (3 x 107) were vortexed vigorously in 2 ml of PBS, and then frozen and thawed four times using liquid nitrogen and a 37°C water bath. Each thaw was followed with vigorous vortexing. The suspension was then sonicated for 20 seconds. The protein concentration of the sonicate was determined as described above, 15 and the suspension stored at -70° until used.

The thawed oocyst suspension was mixed with Freunds Complete Adjuvant for the first injection and Freund's Incomplete Adjuvant for three subsequent boosts. The protein concentrations of each injection in the series were 9 ug, 50 ug, 90 ug, and 90 ug respectively, delivered at four week intervals. The single cat #1959 (designated Queen 4) used for production of antibody to unsporulated oocysts had been orally infected with 100 mouse brain-derived C strain tissue cysts one month before the initial protein injection. Serum obtained from this cat (designated herein as Q4-1959) was analyzed for the presence of antibody specific to T. gondii oocysts by Western blot and immunohistochemistry on a monthly schedule during the injection period.

Immunoscreening the λ gt11:Toxoplasma genomic expression library and isolation of Toxoplasma-specific nucleic acid molecules reactive with antisera to oocysts: Antisera Q4-1959 was used to isolate nucleic acid molecules herein designated OC-1, OC-2, OC-13, OC-14, OC-22, OC-23 as follows: E. coli Y1090 was infected with approximately $5X10^6$ plaque forming units (PFU) of the λ gt11:Toxoplasma genomic expression library, and then evenly spread on 20 LB-amp agarose culture

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plates. The phage were allowed to grow for about four hours at 37°C. The plates were then overlayed with nitrocellulose filters impregnated with 10 mM isopropyl-B-D-thiogalactoside (IPTG) to induce the expression of the recombinant Toxoplasma protein. The induction proceeded for between 4 hours to overnight and then the filters were marked to establish orientation. The filters were removed and, following several washes in TBST (Tris-buffered saline (TBS) + Tween 20: 20 mM Tris, pH 7.5, 150 mM NaCl, 0.5% Tween-20), and an incubation in blocking solution (TBS + 5% powdered milk), incubated with a 1:40 dilution of antisera Q4-1959 for about 3 hours at room temperature or overnight at 4°C. After 3 to 5 washes with TBST the filters were incubated with a 1:1000 dilution of alkaline phospatase (AP) -conjugated goat anti-cat IgG (available from Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD) at room temperature for two hours. The filters were washed two times with TBST and once with TBS. The color indicator was developed in AP buffer (100 mM Tris pH 9, 100 mM NaCl, 5 mM MgCl) containing 0.7% NBT (nitroblue tetrazolium) and 0.3% BCIP (5-bromo-4-chloro-3-indolyl phosphate).

Plaques in the area corresponding to the positive signals were picked into SM buffer (50 mM Tris, pH 7.5, 100 mM NaCl, 8 mM MgSO₄, and 0.01% gelatin) and the phage replated at a lower density. The same screening procedure was repeated three or four times until a pure plaque was isolated. Of the approximately 5X10⁶ plaques screened in this manner, 6 nucleic acid molecules capable of expressing proteins recognized by antisera Q4-1959 were plaque purified.

Characterization of Immunogenic *T. gondii* proteins encoded by nucleic acid molecules selected from the *T. gondii* genomic expression library:

The nucleic acid molecules identified as positive for expression of immunogenic

T. gondii proteins by immunoscreening with antisera Q4-1959 were screened for expression of proteins reactive with intestinal secretions from immune cats. The production of immune intestinal secretions is described in detail in Example 6, below. Prior to being used for screening, pooled intestinal secretions were preabsorbed with E. coli lysates as follows. Individual cultures of E. coli Y1090 cells and XL-1 blue cells

(available from Stratagene) were grown overnight in LB Amp medium at 37°C. The cells were harvested by centrifugation, then resuspended in PBS, pH 7.4. The cell

suspensions were then frozen and thawed 3 times, using a dry ice-acetone bath and a 37°C water bath, then sonicated on ice for 10 minutes. The protein concentrations of the resulting cell lysates were adjusted to approximately 20 mg/ml, then diluted 1:10 in PBS. Fresh nitrocellulose filters (82 mm) were coated with bacterial proteins by immersing them in the diluted E. coli lysates at room temperature for 1 hour. The filters were further incubated in a solution of 4% (w/v) powdered milk in PBS, pH 7.4 for 30 minutes. The filters were then washed with PBS three times for 10 minutes each at room temperature. Pooled immune cat intestinal secretions were diluted 1:20 with 4% (w/v) powdered milk in PBS, pH 7.4. The diluted secretions mixture was incubated with the E. coli lysate-treated filters at room temperature for 1 hour, at a ratio of 20 ml per six 10 filters. The resulting absorbed immune intestinal secretions were used without further dilution to screen nucleic acid molecules identified as positive by immunoscreening as described below. Essentially the same protocol was followed when characterizing the proteins expressed by nucleic acid molecules isolated by immunoscreening with other antisera(as described below). 15

Plaque pure phage identified as positive by immunoscreening were diluted in SM buffer to approximately 50 PFU/3µl. 3 µl of each clone was dropped onto an LB/Amp agar plate which was previously overlayed with top agar containing a 1:20 dilution of a fresh culture of E. coli Y1090 at mid-log growth. The plates were then incubated at 370 C for 5 hours. IPTG-treated nitrocellulose filters were placed on the top agar and incubated for 5 hours. The filters were marked, washed in TBS buffer, pH 8.0 at room temperature for 15 minutes and then blocked with 4% (w/v) powdered milk in TBS for 30 minutes, at room temperature. The filters were incubated with absorbed intestinal secretions at 4°C overnight. All further manipulations were at room temperature. The filters were washed in TBS buffer for 10 minutes, 3 times. The filters were incubated for 2 hours with a 1:300 dilution of horse radish peroxidase (HRP) -conjugated goat anti-cat IgA polyclonal antibody (available from Bethyl Laboratories Inc.) in TBS buffer. The filters were washed in TBS for 10 minutes, 3 times, then incubated with 4-chloro-1naphthol substrate. Clones were judged to be either positive or negative by the intensity of the color reaction relative to wild type phage controls. The results of this assay are summarized in Table 2. Of the six nucleic acid molecules expressing proteins

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recognized by antisera Q4-1959, only OC-1 expressed a protein that was positive for reactivity to immune cat intestinal secretions.

Table 2

Nucleic Acid Molecules Selected with Cat Sera Specific to Unsporulated Oocysts

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IVITY	2		+		۱.			•	•				
REACTIVITY	SERUM		QN	9	QN N	CN		2	QN		2		
 DVAC	IN VIVO		CZ		2		-	2	+		2		
Qa	IN VITRO IN VIVO		CZ	2	QN		+	S		٠	Q		
NOIS	1000	200	9	2	2	2	2	2		2	S		
	EAPRESSION 100	DITCHIS		1		+	+		•	+		•	
		82		•	-	+	-	١	+	7,5	•	+	
	DETECTION	7.7			+	7	-	+	+		+	2+	
	DETE	nce			+		•				,		
		501	-1		+		+	7.	3	+	40		17
MICIO	ORIGINAL	SEQ ID NO DESIGNATION				96-1	600	127	0C-13	14		0C-22	0C-23
		SEQ ID NO				02	1	7 21	74	١	1	82	80

(ND) indicates not done. In the column labeled "Detection", the numbers associated with the positive responses indicate the intestinal secretion (IS) of the expressed product. In all cases, (+) indicates a positive response, (-) a negative response, and "pDVAC" refers to subcloning into and expression from the eukaryotic expression vector pDVACI, as tested in vitro (BHK infected cat gut cells (ICG), uninfected cat gut cells (UCG), tachyzoites (TZ), and bradyzoites (BZ); "Expression" refers to original name for each nucleic acid molecule; "Detection" represent2s the results of RT-PCR assays to assess cDNA from "SEQ ID NO" is the nucleic acid sequence designation for the nucleic acid molecule; "Original designation" is the relative signal strength for each primer assayed with each of the four cDNA samples, i.e., ICG, UCG, TZ, and BZ cDNA, results of subcloning the nucleic acid molecule into one or both of two E coli expression plasmids, pTrCHIS and λ CRO; cells) and in vivo (mice); "Reactivity", indicates specific recognition by cat immune sera (serum) and/or cat immune and are not a comparison between primers. Table 2 Legend:

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Some of the nucleic acid molecules identified as positive by immunoscreening were also assessed for expression of proteins reactive with Mozart II (immune) sera. Reactivity was assessed by spotting the purified phage directly on a lawn of host *E. coli* and inducing the expression of protein encoded by the cloned DNA insert using IPTG-soaked filters, similar to the phage screening protocol. The filters were then probed with the Mozart II sera, in essentially the same manner as was used to select the plaque purified phage identified as positive by immunoscreening. The results of these assays are summarized in Table 2.

The Toxoplasma inserts in λ gt11, herein referred to as λ gt11:Toxoplasma nucleic acid molecules were sequenced either by direct sequencing, or by first subcloning the λ gt11:Toxoplasma nucleic acid molecules into a cloning vector, then sequencing. Direct sequencing of each insert was performed as follows: the Toxoplasma-specific insert in λ gt11 was PCR amplified under standard conditions well known in the art using a λ gt11 forward primer (5' GGTGGCGACGACTCCTGGAG 3') and a λ gt11 reverse primer (5' CCAGACCAACTGGTAATGGTAG 3'), and the major PCR reaction product was separated from the rest of the PCR reaction products on a 1% agarose gel. The band representing the major PCR product was excised, and the gel slice was processed using the QIAquick kit (available from Qiagen Inc., Santa Clarita, CA) according to manufacturer's instructions in order to release the DNA. The isolated DNA fragment was sequenced under standard conditions using an ABI PRISM 377 automated DNA sequencer (available from Applied Biosystems, Foster City, CA). Each of the amplification primers were used separately as sequencing primers to obtain sequence from both directions.

Subcloning, then sequencing, was performed as follows: the Toxoplasmaspecific insert was PCR amplified and gel purified as described above. The purified
DNA was then cloned into a TA cloning vector (available from Invitrogen Corp., San
Diego, CA) according to the manufacturer's instructions, and sequenced under standard
conditions.

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Sequence analysis of nucleic acid molecules selected for expression of proteins recognized by antisera Q4-1959:

The nucleic acid molecules selected for expression of proteins recognized by antisera Q4-1959 were sequenced as described above. BLASTn and BLASTp homology searches were performed on these sequences using the NCBI GenBankTM non-redundant (nr) nucleotide (n) and amino acid (p) databases, and the dbEST (est) database as described above. The results of these searches are summarized in Table 3. Nucleic acid molecule OC-1 was sequenced again and some changes found between the first and second sequence. The resequenced nucleic acid molecule is referred to herein as OC-1-a.

Table.

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	Clone/Match										302-2/8-308	446-345/8-109	590-489/8-109	349-135/129/342	493-418/129-204	137-16/5-126	363-127/192-428	500-364/340-476	356-220/340-476	601-515/383-469	178-106/350-422	241-205/456-492	373-349/468-492	113-249/340-476	257-403/340-486
	Size										553						574			·				574	
HOMOLOGIES	Name										TgESTzz29d08.r1 invivo Bradyzoite cDNA						TgESTzz43d05.s1 TgME49 invivo Bradyzoite							TgESTzz43d05.s1 TgME49 invivo Bradyzoite	
TOP HITS	Gene										1.20E-112 AA531653						AA520213							520213	
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Table 3

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Table 3

²⁸ 5 251--263 72--72 864-1126/55-317 809-868/1-60 454 TgESTzy98f02.r1 TgME49 tachyzoite HOMOLOGIES 4.70E-119 AA037916 4.50E-43 N82635 1.20E-36 N96576 2.20E-36 N82193 TOP HITS 4.60E-128 | W96667 4.50E-43 1 1.20E-36 2.20E-36 # P (N) < 1e-10 1478 381 Size SEQ ID

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Table 3 Legend:

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Results of BLASTn and BLASTp search of the NCBI GenBankTM non-redundant (nr) nucleotide (n) and amino acid (p) databases, and the dbEST (est) database. The algorithm used was as described in S.F. Altschul, W. Gish, W. Miller, E.W. Myers, and D.J. Lipman, J. Mol. Biol. 215, 403-10 (1990) and the NCBI. From left to right: are the sequence identification number (SEQ ID No), the size of the nucleic acid molecule (Size) in either base pairs (bp) or amino acids (aa), the number of hits below the sum probability score of 1^{e-10} (# P(N) < 1e-10), and a section of the hits with the highest homology (HOMOLOGIES). The homologies section is sub-divided to include the sum probability (Score) of the homology, the gene accession number (Gene), the name or identifier of the gene (Name), the size of the gene either in nucleotides, if it is a match in the BLASTn or amino acids if it is in the BLASTp (Size), the range of either nucleotides or amino acids in which a match was identified in the clone versus the match in the database (Clone/Match), the number of identities compared with the range matched (Identities), and the percentage homology of the match (%). A dash (-) indicates the search was done and there were no matches.

RT-PCR analysis of nucleic acid sequences encoding Immunogenic T. gondii

proteins:

The sequence data obtained as described above were used to design unique primers specific to each nucleic acid molecule of the present invention. These primer sequences are listed in Table 4.

Table 4

Nucleic Acid Molecules Primer Sequences

SEQ ID NO.	ORIGINAL DESIGNATION	3	PRIMER SEQUENCE	BASE PAIR NUMBERS
144				
145	Tg-41 (5')	nTG1	CGCTTCTTGTGTCACCTG	118
146	Tg-41 (3')	nTG1	GCACCTTGTTCTCTCTCTCGCC	317-295
147	Tg-45-2T (5')	nTG2	CGAGGAGACGGTGGGAGC	118
148	Tg-45-2T (3')	nTG2	TGCCCAAGATGCCGATCTCTG	289269
149	Tg-50 (5')	nTG4	TCTCCCCATCGACGAAAAC	95-114
150	Tg-50 (3')	nTG4	GCTCATTTCCTCCGCAATTTGG	456435
151	Q2-4 (5')	nTG5	AGCTGGCAGAAATACCAAAGCTC	67-90
152	Q2-4 (3')	nTG5	TGTCGGCAATACTGGGCATG	529510
153	Q2-9 (5')	nTG6	ACTGGAGTGGAAAGTCTGGTTTTG	3760
154	Q2-9 (3')	nTG6	GACGCAGAGAAGAAGAAGAGCC	415393
155	Q2-10 (5')	nTG7	TCCAAAACTGTCTCGTCTCCCC	165186
156	Q2-10 (3')	nTG7	TCTGGATACGCCGTTCCTTTG	305-284
157	Q2-11 (5')	nTG8	GACATCTACCTGTGAGTGAACCAGG	5074
158	Q2-11 (3')	nTG8	GTCAAAACCTTGCCAGCATCTC	475454
159	4499-9 (5')	nTG9	TCCGACTGAATGACTACCTCTTTC	4528
160	4499-9 (3')	nTG9	TCCGACCAAGTCCTCAGTGAAC	537-516
161	4604-2 (5')	nTG10	TGGGCATTTCCTGGAAGAGG	3655
162	4604-2 (3')	nTG10	GAATCCATCTCGTGCAAACGG	378-358
163	4604-3 (5')	nTG11	CAAGACACAGGGAAACGTTGG	102-122
164	4604-3 (3')	nTG11	GAAAGAATCGCACCTCCTCTCC	424-403
165	4604-5 (5')	nTG13	TTTGAGTCTAACCGCCGTATGTC	20-42
166	4604-5 (3')	nTG13	TCAGACGATTCTCCCATTGTACG	216-194
167	4604-10 (5')	nTG15	TCGACTTGGGTCCGATTGTTAG	4364
168	4604-10 (3')	nTG15	GATCTTTTGCGTGACTTTGTCTGC	289-266
169	4604-17 (5')	nTG16	GAAGATGCTTGTCTTGTTCGGTTC	19-42
170	4604-17 (3')	nTG16	GAGGGGTTTCCTTCTTTATTGCC	178-156
171	4604-54 (5')	nTG17	TGTTGGACATCCCGAGCATC	23-42
172	4604-54 (3')	nTG17	GGTCCTTGTTTTTCAGGCGG	472-453
173	4604-62 (5')	nTG18	TCGTGCAGACAGTGAAGCAATG	35-56
174		nTG18		201-281
175	4604-63 (5')	nTG19	CGCAAGTGAGTTTTGGCTTTACC	15-37
176		nTG19	CCTGGAAGAGATATGCAGACAC	389368.
177	4604-69 (5')	nTG21	TCACCGTTCGCTCTTCTTTCTC	12-33
178	4604-69 (3')	nTG21	CGACTGAAGCATGGATTGCC	367-348
179	AMX/I-5 (5')	nTG31	ACATATTCCTGAGGAGGAGTTCCC	82-105
180	AMX/I-5 (3')	nTG31	AACACACCTCCGACGACACCAC	447-426
181	AMX/I-6 (5')	nTG32	CTCGGCTTCTCCACATACAAGG	8-29
182	AMX/I-6 (3')	nTG32	GGATCTAGGCATTTGGGTTTCAC	411-389
183	AMX/I-7 (5')	nTG33	ATCGAAGAAGCTGAAGCGGAG	4-24
184		nTG33	GTGCTTGTCTCTGACGAAACCC	193-172
185	AMX/I-9 (5')	nTG34	TATCATTGTATCCCGTCGTCCC	4768
186	AMX/I-9 (3')	nTG34	TGATGCCTGGATTTGCACAAC	363-343
187		nTG35	CGGATCGCTCTGAGTCTCTTTG	122

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Table 4

Nucleic Acid Molecules Primer Sequences

188	AMX/I-10 (3')	nTG35	AICCIGIOIOIO CONTRA	384362 88110
189	AMI-23 (5')	nTG36	GATCGCTCTGAGTCTCTTIG	
	AMI-24 (5')	nTG37	ACGTGAGGGAGAGAGAGAGAGIGC	21-44
190	AMI-24 (3')	nTG37	TTCATCGTCGCCTCTGATGTCC	347-326
191	AMI-28 (5')	pTG38	TGTAGACAGCGTTTAGGGAGTGC	21-43
192	AMI-28 (3')	DTG38	GTCCTTGGAAGTGCAGAAGCAG	440419
193	AMI-47 (5')	nTG40	AAGCGAGGAAAAGGAGGTGTC	95-115
194	AMI-47 (3')	nTG40	CGGGAAGGTTGGTGATGTCTGTG	252-230
195		nTG41	CCCGAAGACTTTGACCTG	3451
196	OC-1 (5')	nTG41	AGTGGCATAGGAGGCTGG	191174
197	OC-1 (3')	nTG42	GCACCTTCAATGCCACAGGTATC	90112
198	OC-2 (5')	nTG42	TCGTGTGCTTCTCGCTTCTCTG	484-463
199	OC-2 (3')	nTG43	CACTGTCGATCAGAAGAAGGCTTAC	84108
200	0C-13 (5')		GCTCCGTGGGCACATTTTG	367348
201	0C-13 (3')	nTG43	CAGTTTACGAGGTACAAGGCAACAG	933
202	0C-14 (5')	nTG44	GATTGCGTGGGCAGTGTAGAAG	237-216
203	OC-14 (3')	nTG44	TGTTTGTTTCCCCAGTCAACGAC	89111
204	0C-22 (5')	nTG45	CGGAAGAGGTTGTTGGACTCCTTC	570547
205	0C-22 (3')	nTG45	TO A CACACACACACACACACACACACACACACACACACA	62-86
206	0C-23 (5')	nTG46	TGGGGAGAACAGCAGACATCAG	602-581
207	0C-23 (3')	nTG46	TO TO TO TO A TO A TO A TO A TO A TO A	627
208	4CQA11 (5')	nTG49	COACTICCTCCCTC	270-256
209	4CQA11 (3')	nTG49	CGACTTGGTCCGCTC CGGCGGCAACAAATGGGC	1-18
210	4CQA19 (5')	nTG50	GTCCGAGATATGAGGATGCGAC	129108
211	4CQA19 (3')	nTG50	TCAGAGCACCATTGTTGCGAC	39-59
212	4CQA21 (5')	nTG51	TTTGACGCTCAAGTGGAGGCTG	556535
213	4CQA21 (3')	nTG51		615-633
214	4CQA22 (5')	nTG52		810-788
215	4CQA22 (3')	nTG52	CHCHGACIACCHGAGGIGT	283-300
216	4CQA24 (5')	nTG53	AAGGACAAGCCTGGTTTG	1130-1113
217	4CQA24 (3')	nTG53		82-103
218	4CQA25 (5')	nTG54	THE SECOND ACCULATION OF THE CASE	459-438
219	4CQA25 (3')	nTG54	THE TOTAL OF CACCACCACTACT	4567
220	4CQA26 (5')	nTG55		266244
221	4CQA26 (3')	nTG55		1-18
222	4CQA27 (5')	nTG56		174-153
223	4CQA27 (3')	nTG56		129-110
224	4CQA29 (3')	nTG57	TTCAGCGGGTCTTTCCTCAC	3455
225	R8050-2 (5')	nTG58	CAACGAGAAAGATGGAGCTTCG	404-384
226	R8050-2 (3')	nTG58	B AACTTCTTGCACTTGGTCCCG	122
227	R8050-5 (5')	nTG60	AAGCGAGGAAAAGGAGGTGTCTC	95118 250230
228	R8050-5 (3')	DTG60	TGGAAGGTTGGTGATGICIGIG	8-3O
229	R8050-6 (5')	nTG6	TTCCCCCAGGAATTGTTGAAACAG	254-230
230	R8050-6 (3')	nTG6	TACTACCGACAACGTCTCAGTCCTTC	
	M2A1 (5')	nTG6	CGTGCGTCTGTGAGGAAAAGTG	223
231	M2A1 (3')	nTG6		341-320

Table 4

Nucleic Acid Molecules Primer Sequences

233	M2A3 (5')		TTGTTCTCGAACCCGCAGAG	7493
234	M2A3 (3')	nTG64	TGGCAAGAGACCGAATCGTG	235-216
235	M2A4 (5')	nTG65	AAACTTGGCAAAGGGGAACG	4968
236	M2A4 (3')	nTG65	TGCTGTGGAGAATGATGGCTG	483463
237	M2A5 (5')	nTG66	TTTCCGACGAAGCTGCC	2541
238	M2A5 (3')	nTG66	GACTCCAACGAAAGCCTCG	144-126
239	M2A6 (5')	nTG67	GGAAAGGGATAAAGACGCCG	150169
240	M2A6 (3')	nTG67		337314
241	M2A7 (5')	nTG68	CTGCACCATTTCTCACTTCTTGTG	5780
242	M2A7 (3')	nTG68	GCAAAAGCGGACTCGATTCTATTG	192169
243	M2A11 (5')	nTG69		1231
244	M2A11 (3')	nTG69		406385
245	M2A16 (5')	nTG70		195214
246	M2A16 (3')	nTG70		759738
247	M2A18 (5')		TCACGCAACGAACAAGTCCTC	4262
248	M2A18 (3')	nTG71	CCCATTTTTGCTTGGCTTGC	149130
249	M2A19 (5')	nTG72	AGCGGCAAACCAGTTCGTTG	283-302
250	M2A19 (3')	nTG72		558539
251	M2A20 (5')	nTG73		116
252	M2A20 (3')	nTG73		131-109
253	M2A21 (5')	nTG74	L	127-146
254	M2A21 (3')		TCATGTTGGAGGCGTCGTTC	241222
255	M2A22 (5')	nTG75	TGTGCAGTGGAGGACAAATGG	50-70
256	M2A22 (3')	nTG75		284264
257	M2A23 (5')		ATTCTGTGCAAGCCCAGAG	305323
258	M2A23 (3')		CGACCAAGGGTGTTGACCAT	136155
259	M2A24 (5')	nTG77	CTAGGCAAAGAAACACCCATGC	226-247
260	M2A24 (3')	nTG77	CGCTGGAACTCCTGACAC	327-310
261	M2A25 (5')		ACGAAGGAGAGATGCGTTTG	5979
262	M2A25 (3')	nTG78	TGGCTGTTTGGGTTGTCTGG	392-373
263	M2A29 (5')		TCACCGCAGAACTTAACCCG	6281
264	M2A29 (3')	nTG79	CTCGCTTTTCCAGCTTGTCG	249-230

Table 4 Legend:

Primer Sequences to Nucleic Acid Molecules. The original name (Original Designation) and the present name (Name) for each nucleic acid molecule are listed in the second and third columns. Separate 5' and 3' primer sequences are listed for the nucleic acid molecules under Primer Sequence. Identification of each primer sequence as 5' or 3' is shown in the column labeled Original Designation. The location of each primer sequences in its respective nucleic acid molecule is shown in the column. Base Pair Numbers. The sequence identification number for each primer is listed in the first column (Seq ID NO).

The unique primers listed in Table 4 were used in reverse transcriptase-polymerase chain reaction (RT-PCR) assays to assess the expression of the particular nucleic acid sequence in ICG, bradyzoites and tachyzoites. DNA templates were generated from total or poly A+ RNA using an RT-PCT kit (available from Stratagene) according to the manufacturer's instructions. The resulting DNA templates were then amplified by standard PCR reaction. The RT-PCR reactions were performed using RNA isolated from infected cat gut (ICG), bradyzoites (BZ), tachyzoites (TZ), and the appropriate controls (e.g., uninfected cat gut (UCG) RNA). In addition to UCG controls, clone-specific primers were used in PCR reactions using DNA from the following sources: *T. gondii*, mouse cells, cat intestinal cells, and human cells. These results are summarized in Table 2.

Subcloning *T. gondii* nucleic acid molecules encoding Immunogenic *T. gondii* proteins into the expression vector pTrcHisB:

T. gondii nucleic acid molecules encoding immunogenic T. gondii proteins isolated as described above were subcloned into the expression vector pTrcHisB (available from Invitrogen Corp., San Diego, CA). The vector pTrcHisB is designed for expression of fusion proteins in E. coli and purification of proteins encoded by nucleic acid molecules of interest. Expression of fusion proteins from this vector was assessed following induction and subsequent Western blot analysis of the E. coli lysates using both a monoclonal antibody to the T7 phage amino acid tag sequence and the original sera used to select the nucleic acid molecule. The fusion proteins all contain a poly histidine amino acid sequence which was used to purify the fusion proteins using metal chelate chromatography.

Recombinant molecules containing nucleic acid sequences encoding

25 immunogenic *T. gondii* proteins were produced by PCR amplifying plaque purified λ gtl1:Toxoplasma nucleic acid molecules using a λ gtl1 forward primer (5' GGTGGCGACGACTCCTGGAG 3') and a λ gtl1 reverse primer (5' CCAGACCAACTGGTAATGGTAG 3'). Amplifying the Toxoplasma inserts in this way produced DNA fragments with *EcoR* I sites at the junctions between the

30 Toxoplasma insert and the lambda vector. These PCR fragments were then digested with the restriction endonuclease *EcoR* I, gel purified and subcloned into the *EcoR* I-

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cleaved expression vector, pTrcHisB. The resultant recombinant molecules were transformed into DH5a competent cells to form recombinant cells, and assayed for the expression of an immunogenic T. gondii protein. The results of these assays are summarized in Table 2.

The recombinant coils were cultured in enriched bacterial growth medium containing 0.1 mg/ml ampicillin and 0.1% glucose at about 37° C. When the cells reached an OD_{600} of about 0.4-0.5, expression of recombinant proteins was induced by the addition of 0.5 mM isopropyl-B-D-thiogalactoside (IPTG), and the cells were cultured for about 4 hours at about 37° C. Immunoblot analysis of the recombinant cell lysates using a T7 tag monoclonal antibody (available from Novagen Inc., Madison, WI) directed against the fusion portion of the recombinant Toxoplasma fusion protein was used to confirm the expression of the fusion proteins and to identify their size. In addition, the original selecting antisera were used to determine whether the recombinant expression molecule expressed a protein that could be recognized by the sera originally used to isolate the Toxoplasma-specific portion of the recombinant molecule. The results of these immunoblot assays are summarized in Table 2. Of the six nucleic acid 15 molecules selected by immunoscreening with antiserum raised against oocysts (Q4-1959 serum), six were positive by this immunoblot assay.

Example 3:

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This Example discloses a method of isolation of T. gondii nucleic acid molecules encoding immunogenic T. gondii proteins recognized by antisera raised against the initiating stage of T. gondii gametogony: the bradyzoite. This Example further discloses recombinant nucleic acid molecules, proteins and cells of the present invention.

Antibody To Bradyzoites: Purified C strain bradyzoites (3 x 107) from mouse brain tissue cysts were used to generate stage-specific antibody to T. gondii as follows:

T. gondii C-strain tissue cysts containing bradyzoites were passaged in mice by harvesting tissue cysts from chronically infected mice that had been infected, either intraperitoneally with tachyzoites produced in vitro, or by oral gavage with tissues cysts. Between four and eight weeks post-infection, tissue cysts were harvested and used to inoculate naive mice. Harvest was accomplished by dissecting out the brains of infected mice euthanized by inhalation of CO₂. The brains were added to a tube of 30% Dextran

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in HBSS (Hanks Balanced Salt Solution, available from Life Technologies Inc. (Gibco/BRL), Gaithersburg, MD), and placed on ice until further purified. Each tube contained a maximum of 8 brains per 20 ml of 30% Dextran solution. Tissue cysts were purified by homogenizing the brains for 20-30 seconds with a Tissuemizer (available from Tekmar-Dohrmann, Cincinnati, OH). The homogenized brains were centrifuged for 10 minutes at 3.300 g at 4° C. The supernatant was poured off and the pellet was resuspended in 2.0 ml of HBSS. The pellets from multiple tubes were combined and the tissue cysts were counted using a hemacytometer. To produce a new lot of chronically infected mice, tissue cysts purified as described above were diluted in HBSS to a concentration of 100 tissue cysts/ml. Mice were inoculated by oral gavage with 100 µl (10 tissue cysts). After six weeks there were approximately 600 tissue cysts per mouse.

Bradyzoites were purified from tissue cysts by pepsin digestion and passage through a CF-11 cellulose column. Pepsin digestion was initiated by adding approximately 1.0 ml of pepsin digestion fluid (0.5% pepsin, 0.17 M NaCl, and 1.16 M HCl) fluid per 1.0 ml of cyst suspension. The sample was incubated for 10 min in a 37°C waterbath with occasional swirling. After incubation, approximately 0.9 ml of 0.5% sodium carbonate per 1.0 ml of sample was added slowly and with constant gentle mixing. The solution was then centrifuged for 10 minutes at 2,000 rpm. The supernatant was removed and the pellet resuspended in 5.0 ml of Dulbecco's Modified Eagle's Medium.

1.2 g of CF-11 cellulose was added to 50.0 ml of DMEM, and then poured into a 50 ml chromatography column. The column was equilibrated by allowing most of the DMEM to wash out. The pepsin-digested bradyzoites were diluted with 45 ml of DMEM and loaded onto the column. The column was allowed to drip slowly and the flow through was collected. The column was washed with another 50 ml of DMEM and the flow through was again collected. The two 50 ml flow through aliquots were centrifuged at 2,000 rpm for 15 min. The supernatant was carefully removed and the bradyzoite pellet was resuspended in 1ml of sterile PBS buffer. The number of bradyzoites obtained was determined by counting an aliquot using a hemacytometer.

Bradyzoites prepared as described above were lysed in a PBS, 0.001% Triton X-100 solution by freeze-thawing four times in liquid nitrogen and a 37°C water bath. The

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resulting lysate was further treated by sonication for ten, 30 second bursts, while on ice. Following protein determination using a BCA Protein Kit (available from Pierce Biochemicals, Rockford, IL), the bradyzoite lysate was mixed with Freunds Complete and Freunds Incomplete Adjuvants for the first and subsequent (booster) injections respectively. The first injection of rabbit #2448 contained 46 mg of soluble protein, and the two following boosts contained 6 ug of soluble protein each. Injections were given subcutaneously at four week intervals, and serum, designated 2448, was collected every three weeks.

Antiserum 2448 was used to isolate nucleic acid molecules herein designated BZ1-2, BZ1-3, BZ1-6, BZ2-3, BZ2-5, BZ3-2, BZ4-3 and BZ4-6 as follows: *E. coli* Y1090 was infected with approximately 2X10⁵ PFU and then evenly spread on 4 LB-amp agarose culture plates. The rest of the screening procedure was as described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:200 dilution, and the secondary antibody was a 1:1000 dilution of AP-conjugated goat anti-rabbit IgG. Of the 2X10⁵ plaques screened in this manner, 8 nucleic acid molecules capable of expressing proteins recognized by antisera 2448 were plaque purified.

Characterization of Immunogenic T. gondii proteins encoded by nucleic acid molecules selected from the T. gondii genomic expression library:

The nucleic acid molecules identified as positive for expression of Toxoplasma stage-specific antigenic proteins by immunoscreening with antisera 2448 were screened for expression of proteins reactive with intestinal secretions from immune cats, as described above. The results of this assay are summarized in Table 5. None of the 8 nucleic acid molecules expressing proteins recognized by antisera 2448 were positive for reactivity to immune cat intestinal secretions in this assay.

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Table 5

Nucleic Acid Molecules Selected with Rabbit Sera Specific to Bradyzoites

	ORIGINAL										
SEQ ID NO	DESIGNATION		DETECTION	TION		EXPRESSION	SION	ρDΛ	pDVAC	REACTIVITY	TIVITY
		921	SON	TZ	BZ	pTrCHIS	λCRO	IN VITRO	IN VIVO	SERUM	<u>S</u>
38	BZ1-2	QN	QN	ON	ND	9	ND	QN	ΠN	QΝ	,
40	BZ1-3	2	Q	QN	ND	QN	ND	QN	QN	S	
42	BZ1-6	2	QN	QN	ND	ON	QN	Q	Q	2	
44	BZ2-3	9	Q	QN	ND	QN	ND	QN	Q.	₽	
46	BZ2-5	9	QN	QN	ND	QN	QN	QN	DN	2	
48	BZ3-2	9	QN	QN	ND	QN	ND	QN	QN	2	
20	BZ4-3	QN	QN	QN	ND	QN	ND	QN	QN	Q	.
52	BZ4-6	QN	ND	QN	QN	QN	ND	QN	QN	QN	

Table 5 Legend: See Legend for Table 2.

Sequence analysis of nucleic acid molecules selected for expression of proteins recognized by antisera 2448:

The nucleic acid molecules selected for expression of proteins recognized by antisera 2448 were sequenced as described above. BLASTn and BLASTp homology searches were performed on these sequences using the NCBI GenBank™ non-redundant (nr) nucleotide (n) and amino acid (p) databases, and the dbEST (est) database as described above. The results of these searches are summarized in Table 3. Nucleic acid molecule BZ1-2 was sequenced again and some changes found between the first and second sequence. The resequenced nucleic acid molecule is referred to herein as

BZ2-1-a. 10

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This Example discloses a method of isolation of T. gondii nucleic acid molecules Example 4: encoding immunogenic T. gondii proteins recognized by rabbit antisera raised against infected cat gut. This Example further discloses recombinant nucleic acid molecules, proteins and cells of the present invention.

Production of rabbit antisera to infected cat gut: A pregnant female cat (designated Queen 2) (available from Liberty Laboratories, Liberty Corners, NJ) was maintained in isolation and allowed to come to term. The kittens (4) were housed with the mother and nursed normally throughout the protocol. At day seven post-partum, one kitten was selected as the control and its intestine harvested as described below. The remaining kittens were infected orally with 5000 mouse brain-derived tissue cysts of the T. gondii strain C, by dripping a solution of the tissue cysts in 1 ml of PBS down the back of their throats. The infected kitten intestines were obtained and processed on day 7 post-infection. The Queen 2 was also infected orally at the same time and in a similar fas2hion using 100 tissue cysts of T. gondii C strain. 25

In order to obtain fresh intestine, the following procedure was used for both the control and infected animals. A kitten was first anesthetized by placing it in an inhalation chamber which was flooded with both isoflurane and oxygen until the animal was anesthetized. The kitten was then euthanized with an intravenous injection of commercial pentobarbital euthanasia solution at the recommended dose (88 mg/kg). The animal was immediately dissected to expose the small intestine. This was removed by

excisions at the anterior junction with the stomach and the posterior junction with the large intestine. The intestine was opened by a single cut from the anterior to the posterior end, exposing the mucosal surface. The gut was then dipped sequentially into three separate washing baths containing cold HBSS (Hanks buffered saline solution) (available from Life Technologies Inc. (Gibco/BRL), Gaithersburg, MD). The intestine was then placed flat on a chilled laminated sterile surface with the mucosal layer up. A single piece of dry nitrocellulose (BA85, available from Schleicher and Schuell Inc., Keene, NH) the length of the intestine, ranging in size from 40 to 70 cm long (this varied with the animal) and 5mm wide, was carefully placed lengthwise on the mucosal surface of the intestine to obtain an impression smear of the villus epithelial cells. After the nitrocellulose strip became wet (approximately 30 seconds after application), the strip was carefully lifted off and allowed to air dry. The orientation of the anterior and posterior ends of the intestine and strip were noted. Forty biopsy samples, approximately 4 mm by 4 mm sections, were then taken from random positions throughout the length of the intestine, and immediately fixed in either methanol or gluteraldehyde, and maintained for further histological analysis. The intestine was then cut into ten equal sections, and each section placed in a separate bag, labeled and quick frozen in a dry ice and acetone bath. The intestinal sections were maintained at -70° until further processing.

Sections of the cat gut which contained *T. gondii* were identified using PCR analysis of the DNA captured by the nitrocellulose lift with primers specific to the *T. gondii* α-tubulin gene. The presence of *T. gondii* parasite infection was confirmed by histological analysis of the biopsy sections. Portions of *T. gondii*-positive cat gut sections were then prepared as follows for subsequent injections into rabbits to produce antibody directed toward major epitopes from *T. gondii* gametogenic stages. The same methods were also used to produce antibody in cats to infected cat gut preparations, as herein described (in Example 5). A piece of intestine approximately 2 mm by 20 mm was cut from five frozen sections of infected cat gut material. The pieces were maintained at 4°C, laid flat and the mucosal layer carefully scraped from the intestine wall and muscle layers using a razor blade. This material was then minced and placed in 5 ml of sterile PBS containing 1% nystatin, 10 μg/ml gentamicin, and 1%

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penicillin/streptomycin in a conical centrifuge tube. The mixture was brought through 4 cycles of a freeze-thaw treatment using liquid nitrogen and a 37°C waterbath. The sample was vortexed between each cycle. The sample was placed on ice and then sonicated using a microtip for 20 seconds followed by 20 seconds on ice. This was repeated four times. The suspension was divided among four Eppendorf tubes and centrifuged (Eppendorf 5415C centrifuge, available from Brinkmann Instruments Inc., Westbury, NY) at maximum speed for 30 minutes at 4°C. The supernatant was then put through a 0.22 micron filter and a protein determination performed using the BCA Protein Determination Kit (available from Pierce Chemical Co., Rockford, IL) according to the manufacturer's protocol. The sample was stored as small aliquots at -70°C until used.

Polyclonal antisera against infected cat gut (ICG) antigens (also herein referred to as anti-ICG antiserum, or anti-ICG antibody) were prepared by immunization of New Zealand White rabbits with infected cat gut tissue protein as follows. Six rabbits were injected with the solubilized cat gut material; two rabbits (designated #4603 and #8049) were injected with solubilized material from uninfected cat gut, and 4 rabbits (designated #4604, #4499, #8050, and #8051) were injected with solubilized material from infected cat gut material. For the first injection, 0.5 mg of soluble protein, prepared as described above, was brought to 0.5 ml and mixed with an equal volume of Freunds Complete Adjuvant. This solution was delivered sub-cutaneously (SQ). The second injection, two weeks later, was identical to the first, except Freunds Incomplete Adjuvant was used. A third injection, twelve weeks after the first injection, was similar to prior injections except that the total amount of protein injected was 1.5 mg. The animals were pre-bled prior to the first immunization and were bled at approximately monthly intervals to monitor antibody responses. The blood was allowed to clot at room temperature and serum obtained by centrifugation. The sera were evaluated for the presence of antibody specific to T. gondii by both Western blot analysis using tachyzoite lysates and by indirect immunofluorescent antibody assay (section IFA) using histological sections obtained from infected cat intestine.

The rabbit antisera were preabsorbed to uninfected cat gut material prior to use in immunoscreening, either by absorbing the antisera to Sepharose beads to which

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solubilized uninfected cat gut material had been covalently linked, or by absorbing the antisera to nitrocellulose sheets to which uninfected cat gut protein was bound. Western analysis demonstrated that greater than 98% of the serum reactivity to uninfected cat gut was removed by preabsorption to the column. The remaining (unabsorbed) sera showed reactivity towards *T. gondii* tachyzoite lysates. The unabsorbed sera were used to screen the Toxoplasma genomic library.

Antisera 4604 was used to isolate nucleic acid molecules herein designated 4604-2, 4604-3, 4604-5, 4604-10, 4604-17, 4604-54, 4604-62, 4604-63 and 4604-69 as follows: Two separate immunoscreens were performed with this antisera, and Toxoplasma-specific nucleic acid molecules were isolated form each screen. In the first screen, *E. coli* Y1090 was infected with approximately 5X10⁴ PFU and then evenly spread on 10 LB-amp agarose culture plates. In the second screen, *E. coli* Y1090 was infected with approximately 1.5X10⁶ PFU and then evenly spread on 12 LB-amp agarose culture plates. The rest of the screening procedure was as described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:500 dilution, and the secondary antibody was a 1:500 dilution of AP-conjugated goat anti-rabbit IgG. Of the approximately 1.5X10⁶ plaques screened in this manner, 15 nucleic acid molecules capable of expressing proteins recognized by antisera 4604 were plaque purified.

Antisera 4499 was used to isolate nucleic acid molecule 4499-9 as follows: *E. coli* Y1090 was infected with approximately 5X10⁴ PFU and then evenly spread on 10 LB-amp agarose culture plates. The rest of the screening procedure was as described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:200 dilution, and the secondary antibody was a 1:500 dilution of AP-conjugated goat anti-rabbit IgG. Of the 5X10⁴ plaques screened in this manner, 2 nucleic acid molecules capable of expressing proteins recognized by antisera 4499 were plaque purified.

Antisera R8050 (rabbit antisera raised against infected cat gut) was used to isolate nucleic acid molecules herein designated R8050-2, R8050-5, and R8050-6 as follows: *E. coli* Y1090 was infected with approximately 5X10⁶ PFU and then evenly spread on 10 LB-amp agarose culture plates. The rest of the screening procedure was as

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described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:200 dilution, and the secondary antibody was a 1:1000 dilution of AP-conjugated goat anti-rabbit IgG (available from antibody was a 1:1000 dilution of AP-conjugated goat anti-rabbit IgG (available from Kirkegaard Perry Laboratories). Of the 5X10° plaques screened in this manner, 4 nucleic Kirkegaard Perry Laboratories). Of the 5X10° plaques screened by antisera R8050 were plaque acid molecules capable of expressing proteins recognized by antisera R8050 were plaque purified.

Selected nucleic acid molecules identified by screening for the expression of proteins recognized by rabbit anti-ICG antisera were subcloned and sequenced as described in Example 2. The results of assays to characterize the isolated nucleic acid molecules are summarized in Table 6.

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Table 6

REACTIVITY SERUM 22222222 IN VITRO IN VIVO 222222 9999 Nucleic Acid Molecules Selected with Rabbit Sera Specific to Infected Cat Gut **pDVAC** 999999999 ACRO 운 9 + **EXPRESSION** pTrCHIS 9999 999 **BZ** 5+ + + + + DETECTION SON DESIGNATION ORIGINAL 4604-63 4604-10 4604-5 4604-17 4604-54 4604-62 4604-2 4604-3 4499-9 SEQ ID NO 19 23 23 26 26 26 32 33 38

2 2 2 원원 999 + 2+ 5+ R8050-5 R8050-6 R8050-2 105

Table 6 Legend: See Legend for Table 2.

Sequence analysis of nucleic acid molecules selected for expression of proteins recognized by rabbit anti-ICG antisera 4604, 4499 and R8050:

Nucleic acid molecules 4604-2, 4604-3, 4604-5, 4604-10, 4604-17, 4604-54,
4604-62, 4604-6, 4604-69, R8050-2, R8050-5, and R8050-6 were sequenced as
described above. These nucleic acid molecules were sequenced as described above.
BLASTn and BLASTp homology searches were performed on these sequences using the
NCBI GenBankTM non-redundant (nr) nucleotide (n) and amino acid (p) databases, and
NCBI GenBankTM non-redundant (nr) nucleotide (n) these searches are
the dbEST (est) database as described above. The results of these searches are
summarized in Table 3., as described above. The results of these searches are

The sequence data described above were used to design unique primers specific to each nucleic acid molecule of the present invention. These primer sequences are listed in Table 4. The unique primers listed in Table 4 were used in reverse transcriptase-polymerase chain reaction (RT-PCR) assays to assess the expression of the particular nucleic acid sequence in ICG, bradyzoites and tachyzoites. The results of these assays are summarized in Table 6.

T. gondii nucleic acid molecules encoding immunogenic T. gondii proteins isolated by immunoscreening with rabbit anti-ICG antiserum were subcloned into either or both of two expression vectors: pTrcHisB (as described above) or Prcro/T2ori/RSET-B (described below). Expression of the fusion proteins from these vectors, and purification of their expressed fusion proteins, were as described above. The results of assays for the expression of recombinant immunogenic T. gondii proteins from these expression vectors is summarized in Table 6.

Recombinant nucleic acid molecules and protein molecules including sequences
25 encoding T. gondii antigenic proteins and sequences from the vector Prcro T2ori/RSETB: Recombinant molecules containing T. gondii nucleic acid molecules operatively
linked to lambda phage transcriptional control sequences and to a fusion sequence
encoding a poly-histidine segment, were produced in the following manner. T. gondii
DNA fragments in λ gt11 were PCR amplified from nucleic acid molecules herein
designated 4499-9, 4604-2, 4604-3, 4604-5, 4604-10, 4604-17, 4604-54, and 4604-69,
designated 4499-9, 4604-2, 4604-3, 4604-5, 4604-10, 4604-17, 4604-54.

were produced by digesting the PCR product with EcoR I, gel purifying the resulting fragment, and subcloning into expression vector PRcro/T2ori/RSET-B (also referred to herein as λ CRO) that had been cleaved with EcoR I and gel purified. Expression vector PRcro/T2ori/RSET-B contains the following nucleotide segments: An about 1990-bp Pvu II to Aat II fragment from pUC19 containing the ampicillin resistance gene and E. coli of replication; an about 1000-bp Pvu II to Bgl II fragment from pRK248cIts (available from American Type Culture Collection, Rockville, MD) containing lambda transcriptional regulatory regions (including the gene encoding clis, the promoter p_R, and a sequence encoding 22 amino acids of the cro protein); an about 60-bp BgI II to Xba I 10 fragment from pGEMEX-1 (available from Promega Corp.) which contains the T7 promoter; an about 166-bp Xba I to EcoR I fragment from pRSET-B (available from Invitrogen, San Diego CA) which contains sequences encoding the T7-S10 translational enhancer, the His, fusion, the 14-amino acid S10 leader fusion, and an enterokinase cleavage site as well as the multiple cloning site; and an about 210-bp EcoR I to Aat II 15 fragment containing synthetic translational and transcription termination signals including the T₁ translation terminators in all three reading frames, an RNA stabilization sequence from Bacillus thurengiensis crystal protein and the T2 rho-independent transcription terminator from the trpA operon. Expression vector PRcro/T2ori/RSET-B contains the following nucleotide segments. An about 1990-bp PvuII to AatII fragment 20 from pUC19 containing the ampicillin resistance gene and E. coli of replication; an about 1000-bp PvuII to BgIII fragment from pRK248clts (available from American Type Culture Collection, Rockville, MD) containing lambda transcriptional regulatory regions (including the gene encoding cl¹⁶, the promoter p_R, and a sequence encoding 22 amino acids of the cro protein); an about 60-bp Bg/II to XbaI fragment from pGEMEX-1 (available from Promega Corp., Madison WI) which contains the T7 promoter; an about 25 166-bp Xbal to EcoRI fragment from pRSET-B (available from Invitrogen Corp., San Diego CA) which contains sequences encoding the T7-S10 translational enhancer, the His, fusion, the 14-amino acid S10 leader fusion, and an enterokinase cleavage site as well as the multiple cloning site; and an about 210-bp EcoRI to AatII fragment 30 containing synthetic translational and transcription termination signals including the T₁ translation terminators in all three reading frames, an RNA stabilization sequence from

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Bacillus thurengiensis crystal protein and the T_2 rho-independent transcription terminator from the trpA operon.

The resulting recombinant molecules were transformed into E. coli to form recombinant cells, using standard techniques as disclosed in Sambrook et al., ibid.

The recombinant cells were cultured in shake flasks containing an enriched bacterial growth medium containing 0.1 mg/ml ampicillin and 1% glucose at about 32°C. When the cells reached an OD₆₀₀ of about 0.6, expression of the Toxoplasma antigen was induced by quickly adjusting the temperature to 42°C and continuing cultivation of the cells for about 2 hours. Protein production was monitored by SDS PAGE of recombinant cell lysates, followed by immunoblot analysis using standard techniques as described herein and as known in the art. The results of these assays are summarized in Table 6.

The antisera used to originally isolate each Toxoplasma-specific nucleic acid molecule (i.e., either antiserum 4604, or antiserum 4499) was used to identify recombinant proteins in E .coli extracts as follows. The material in crude extracts from E .coli were separated by running 5 μ g protein per lane on a 12-well 10% Tris-glycine SDS-PAGE gel at 200 volts for 1 hour, and then transferred to nitrocellulose membranes by standard methods. After transfer, the membranes were blocked in 5% (w/v) dry milk for 1 hr at 37°C. The membranes were then incubated with a 1:200 dilution in Tris buffered saline of the sera originally used to select the nucleic acid molecule encoding Toxoplasma-specific portion of the fusion protein. After 1 hr incubation at room temperature, the blots were washed, and antibody binding resolved using a secondary antibody bound to a substrate for a color indicator. Using the original selecting antibody, immunoblot analysis of E. coli lysates identified fusions proteins at or near the predicted molecular weight of the recombinant fusion protein. The results of these assays are summarized in Table 6.

Histidine tagged fusion proteins were purified from cell lysates as follows. Cell cultures containing nucleic acid molecules of the present invention inserted into either pTrcHisB or λ CRO were grown to an OD₆₀₀ of approximately 0.4 to 0.5. The cultures were induced with IPTG, and the cells harvested 4 hours later. Ten ml of cell culture was centrifuged at 3000 rpm on a table top centrifuge and the protein isolated according

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to the manufacturer's instructions using a Ni-NTA Spin Kit (available from Qiagen Inc.). Protein purification was monitored by SDS PAGE followed by Coomassie Blue staining of the column eluate fractions. Recombinant cells including recombinant molecules 4499-9, 4604-2, 4604-3, 4604-54, and 4604-69 produced proteins that were able to bind to a T7 tag monoclonal antibody (available from Novagen Inc., Madison, WI) directed against the fusion portion of the recombinant fusion protein.

Recombinant nucleic acid molecules and protein molecules including sequences encoding *T. gondii* antigenic proteins and sequences from the vector pTrcHisB:

Recombinant nucleic acid molecules including sequences encoding T. gondii antigenic proteins and sequences from the vector pTrcHisB were produced as described in Example 2. In brief, T. gondii DNA fragments in λ gtl1 were PCR amplified from nucleic acid molecules herein designated 4604-62, 4604-63, R8050-2, R8050-5, and R8050-6, using the λ gtl1 forward and reverse primers herein described. The resulting recombinant molecules were transformed into E. coli to form recombinant cells 4604-62, 4604-63, R8050-2, R8050-5, and R8050-6. Immunoblot analysis of the recombinant cell lysates using a T7 tag monoclonal antibody (available from Novagen Inc., Madison, WI) directed against the fusion portion of the recombinant Toxoplasma fusion protein was used to confirm the expression of the fusion proteins and to identify their size. Of the six nucleic acid molecules selected by immunoscreening with rabbit anti-ICG antiserum that were subcloned into the expression vector pTrcHisB, 15(4604-62, R8050-2, and R8050-5) were positive by this immunoblot assay. The results of these assays are summarized in Table 6.

Example 5:

This Example discloses a method of isolation of *T. gondii* nucleic acid molecules encoding immunogenic *T. gondii* proteins recognized by cat antisera raised against infected cat gut. This Example further discloses recombinant nucleic acid molecules, proteins and cells of the present invention.

Preparation of cat antibody against infected cat gut: Preparation of infected cat gut material and production of anti-ICG antisera in cats was performed essentially as herein described for production of rabbit anti-ICG antiserum. Polyclonal cat antisera against infected cat gut (ICG) antigens (also herein referred to as anti-ICG antiserum or

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antisera, or anti-ICG antibody) were prepared by immunization of cats as follows. Three cats were injected with cat gut material. One cat (#AME5) was injected with material from uninfected cat gut material and two cats (#AMI4, #AMX1) were injected with material from infected cat gut preparations. The same injection, boost and bleed regimen and antigen preparation were used for cats as was used for rabbits, described above. Like the rabbit antisera, the cat antisera were preabsorbed to uninfected cat gut material prior to use in immunoscreening.

Anti-sera AMI was used to isolate nucleic acid molecules herein designated AMI-23, AMI-24, AMI-28, and AMI-47 as follows: E. coli Y1090 was infected with approximately 5X106 PFU and then evenly spread on 10 LB-amp agarose culture plates. The rest of the screening procedure was as described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:200 dilution, and the secondary antibody was a 1:1000 dilution of AP-conjugated goat anti-cat IgG. Of the 5X106 plaques screened in this manner, 6 nucleic acid molecules capable of expressing proteins recognized by antisera AMI were plaque purified.

Anti-sera AMX/I was used to isolate nucleic acid molecules herein designated 15 AMX/I-5, AMX/I-6, AMX/I-7, AMX/I-9, and AMX/I-10 as follows: E. coli Y1090 was infected with approximately 5X106 PFU and then evenly spread on 12 LB-amp agarose culture plates. The rest of the screening procedure was as described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:200 dilution, and the secondary antibody was a 20 1:1000 dilution of AP-conjugated goat anti-cat IgG. Of the 5X106 plaques screened in this manner, 6 nucleic acid molecules capable of expressing proteins recognized by antisera AMX/I were plaque purified. The results of this immunoscreen are summarized in Table 7. 25

Table 7

Nucleic Acid Molecules Selected by Cat Serum Specific to Infected Cat Gut

SEQ ID NO	ORIGINAL SEQ ID NO DESIGNATION		DETECTION	TION		EXPRESSION	NOI	ΛQd	pDVAC	REAC	REACTIVITY
		၅၁	ອວດ	Z1	BZ	pTrCHIS	лско	IN VITRO IN VIVO	IN VIVO	SERUM	SI
54	AMX/I-5	+	•	+	+	+	+	QN	QN	QN	+
55	AMX/I-6	2+		2+	+	QN	+	ΩN	QN	2	
57	AMX/I-7	2+	•	•	+	QN	+	QN	QN	Q	
59	AMX/I-9	2+	•	+	+	+	QN	QN	QN	Q.	!
61	AMX/I-10	+	+	•			+	QN	QN	Q	
63	AMI-23	+	+	•	•	Q.	ND	QN	DN	Q	,
65	AMI-24	+	•	+	5 ‡	+	9	QN	QN	Q	 -
29	AMI-28	+	•	+	2+	Ą	Q	QN	QN	2	•
89	AMI-47	•	,	+	•	+	Q	QN	QN	QN	,

Table 7 Legend: See Legend for Table 2.

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Selected nucleic acid molecules identified by screening for the expression of proteins recognized by cat anti-ICG antisera were subcloned and sequenced as described in Example 2.

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Sequence analysis of nucleic acid molecules selected for expression of proteins recognized by cat anti-ICG antisera AMI and AMX/I:

The nucleic acid molecules isolated using antisera AMI or AMX/I were sequenced as described above. BLASTn and BLASTp homology searches were performed on these sequences using the NCBI GenBankTM non-redundant (nr) nucleotide (n) and amino acid (p) databases, and the dbEST (est) database as described above. The results of these searches are summarized in Table 3.

The sequence data described above were used to design unique primers specific to each nucleic acid molecule of the present invention. These primer sequences are listed in Table 4. The unique primers listed in Table 4 were used in reverse transcriptase-polymerase chain reaction (RT-PCR) assays to assess the expression of the particular nucleic acid sequence in ICG, bradyzoites and tachyzoites. The results of these assays are summarized in Table 7.

T. gondii nucleic acid molecules encoding immunogenic T. gondii proteins isolated by immunoscreening with cat anti-ICG antiserum (antiserum AMI or AMX/I) were subcloned into either or both of two expression vectors: pTrcHisB or Prcro/T2ori/RSET-B (as described above). Expression of the fusion proteins from these vectors, and purification of their expressed fusion proteins, were as described above.

Recombinant nucleic acid molecules, protein molecules and cells including sequences encoding *T. gondii* antigenic proteins and sequences from the vector Prcro/T2ori/RSET-B:

Recombinant molecules containing *T. gondii* nucleic acid molecules operatively linked to lambda phage transcriptional control sequences and to a fusion sequence encoding a poly-histidine segment in the vector Prcro/T2ori/RSET-B, were produced essentially as described above, resulting in the production of recombinant molecules. The resulting recombinant molecules were transformed into *E. coli* to form recombinant cells using standard techniques as disclosed in Sambrook et al., *ibid.* Assays for the

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expression of an immunogenic *T. gondii* fusion protein by these cells were performed as described above, and the results are summarized in Table 7.

Recombinant nucleic acid molecules, protein molecules and cells including sequences encoding *T. gondii* antigenic proteins and sequences from the vector pTrcHisB:

Recombinant nucleic acid molecules including sequences encoding T. gondii antigenic proteins and sequences from the vector pTrcHisB were produced as described in Example 2. In brief, T. gondii DNA fragments in λ gt11 were PCR amplified from nucleic acid molecules herein designated AMX/I-5, AMX/I-9, AMI-24 and AMI-47 using the λ gt11 forward and reverse primers herein described. The resulting recombinant molecules were transformed into E. coli to form recombinant cells AMX/I-5, AMX/I-9, AMI-24 and AMI-47. Immunoblot analysis of the recombinant cell lysates using a T7 tag monoclonal antibody (available from Novagen Inc., Madison, WI) directed against the fusion portion of the recombinant Toxoplasma fusion protein was used to confirm the expression of the fusion proteins and to identify their size. The results of this immunoblot analysis are summarized in Table 7.

Example 6:

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This Example discloses a method of isolation of *T. gondii* nucleic acid molecules encoding immunogenic *T. gondii* proteins recognized by cat immune sera. This

Example further discloses recombinant nucleic acid molecules, proteins and cells of the present invention.

Production of cat immune sera:

Eight specific-pathogen free (SPF) cats (available from Liberty Laboratories, Liberty Corners, NJ), ages 8-10 months, were randomly assigned to two groups; Group 1, n = 5 and Group 2, n = 3 (the uninfected control group). Before the initiation of any studies with these animals, serum samples were taken from each and tested for reactivity to solubilized tachyzoites. Each animal was seronegative for *T. gondii* by standard Western and ELISA analysis using solubilized tachyzoites as the antigen. This serum also served as the pre-bleed in subsequent studies. Feces from each animal were analyzed for the presence of shed *T. gondii* oocysts using flotation by sugar solution centrifugation followed by microscopic examination. Food was removed from both

groups fourteen hours prior to Day 0, and on the day prior to all sample collections. On Day 0 the cats in Group 1 were orally inoculated by syringe at the back of the throat with 1000 mouse brain derived T. gondii tissue cysts of the Mozart strain. This strain represents an isolate from a cat which presented with Toxoplasmosis at the Veterinary Teaching Hospital, Colorado State University, in 1992. The Group 2 cats were not infected.

The Group 1 cats were housed in individual stainless steel cages in an infectious disease isolation unit. The feces from each animal were collected every day for the first fourteen days post infection (PI) and weekly thereafter until parasite challenge. The feces were analyzed for the presence of shed T. gondii oocysts. Five milliliters of whole blood was collected from each animal by jugular venipuncture on the following days post primary infection: 3, 7, 10, 14, 21, 28, 42, 56, 70, 84, 112, 140, 143, 147, 154, 161, 168, and 182.

On day 140 post primary infection, all Group 1 cats were orally challenged with 1000 mouse brain-derived tissue cysts of the Mozart strain. Fecal samples were collected and monitored for the excretion of oocysts for thirty days post challenge (PC). The cats were then bled as before on days: 3, 7, 14, 21, 28, and 42 post challenge.

In addition to the serum samples collected on the bleed dates, both salivary secretions and intestinal secretions were obtained at weeks 0, 1, 2, 4, 8, 10, 16, 20, 21, 22, 23, 24, and 26. These samples were obtained by first anesthetizing each animal with 20 an injection of thiobarbiturate, then intubating the animals and maintaining them with halothane and oxygen. Approximately 0.1 ml of saliva was collected into an equal volume of 0.1 M EDTA. The intestinal secretions were obtained from the upper portion of the small intestine using an endoscope fitted with medical tubing which allowed suction of intestinal fluid. Intestinal secretions were diluted 1:1 with sterile 0.9% NaCl 25 and centrifuged at 10,000 X g for 5 minutes in an Eppendorf centrifuge. The secretions were stored at -70°C until use. Pooled secretions included equal aliquots from all five immune animals from week 20 through 26 post infection. These pooled secretions were used to test the reactivity of intestinal secretions from immune cats to proteins expressed by nucleic acid molecules of the present invention.

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All Group 1 animals shed oocysts in their feces during the primary infection and all seroconverted as assessed by Western blot analysis using tachyzoite lysates as the antigen. None of these animals shed oocysts when challenged, and were therefore considered immune. The sera from the immune animals was pooled, and is referred to herein as Mozart II antiserum or antisera, or as immune antiserum or antisera.

Mozart II antisera was used to isolate nucleic acid molecules herein designated 4CQA-7, 4CQA-11, 4CQA-19, 4CQA-21, 4CQA-22, 4CQA-24, 4CQA-25, 4CQA-26, 4CQA-27, and 4CQA29 as follows: *E. coli* Y1090 was infected with approximately 8.3X10⁵ PFU and then evenly spread on 13 LB-amp agarose culture plates. The rest of the screening procedure was as described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:80 dilution, and the secondary antibody was a 1:50 dilution of monoclonal mouse anti-cat α chain (available from Serotec, Oxford, England) and the tertiary antibody was a 1:1000 dilution of AP-conjugated goat anti-mouse IgG (Kirkegaard Perry Laboratories). Of the 8.3X10⁵ plaques screened in this manner, 13 nucleic acid molecules capable of expressing proteins recognized by Mozart II antisera were plaque purified. The results of assays to characterize these nucleic acid molecules are summarized in Table 8.

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Table 8

 $\overline{\infty}$ REACTIVITY SERUM ONIN NI 9999999999 용 9999999999 Nucleic Acid Molecules Selected with Immune Cat Sera in Screens II and III pDVAC IN VITRO 99999999 9 9999999999 ICRO **EXPRESSION** 원일일 999999999 PTrCHIS 용 + 9999999999 **BZ** 身 # 4 # # 4 2+ 2 ð 9 + 2 2+ 5 5 3 5 9 DETECTION N 2 + nce 9 9 90 ₽ ‡ 5+ 3+ 9 + + + DESIGNATION ORIGINAL 4CQA-19 4CQA-21 4CQA-22 4CQA-24 4CQA-11 4CQA-26 4CQA-25 4CQA-27 4CQA-29 4CQA-7 M2A-16 Tg-45 Tg-50 M2A-11 Tg-41 M2A-2 M2A-5 M2A-3 M2A-1 M2A-4 M2A-6 M2A-7 SEQ ID NO 85 89 89 93 95 99 101 109 111 113 115 က S 119 121 123 125 125 11/

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M2A-18

Table 8

2222222 Nucleic Acid Molecules Selected with Immune Cat Sera in Screens II and III 9999999 9999999 M2A-20 M2A-21 M2A-22 M2A-23 M2A-24 M2A-25 M2A-25 M2A-19 129 131 132 136 139 141 143

Table 8 Legend: See Legend for Table 2.

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In addition to the immunoscreen described above, Mozart II antisera was used in another immunoscreen to isolate nucleic acid molecules herein designated M2A1, M2A2, M2A3, M2A4, M2A5, M2A6, M2A7, M2A11, M2A16, M2A18, M2A19, M2A20, M2A21, M2A22, M2A23, M2A24, M2A25, and M2A29 as follows: *E. coli* Y1090 was infected with approximately 1X106 PFU and then evenly spread on 10 LB-amp agarose culture plates. The rest of the screening procedure was as described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:50 dilution, and the secondary antibody was a 1:200 dilution of AP-conjugated goat anti-cat IgA (available from Bethyl Laboratories Inc., Montgomery, Texas). Of the 1X106 plaques screened in this manner. 18 nucleic acid molecules capable of expressing proteins recognized by Mozart II antisera were plaque purified. The results of assays to characterize these nucleic acid molecules are summarized in Table 8.

Mozart II antisera was also used in yet another immunoscreen to isolate nucleic acid molecules herein designated Tg-41, Tg-45, and Tg-50 as follows: *E. coli* Y1090 was infected with approximately 1X106 PFU and then evenly spread on 12 LB-amp agarose culture plates. The rest of the screening procedure was as described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:50 dilution, and the secondary antibody was a 1:200 dilution of AP-conjugated goat anti-cat IgA Fc. Of the 1X10 plaques screened in this manner, 4 nucleic acid molecules capable of expressing proteins recognized by Mozart II antisera were plaque purified. The results of assays to characterize these nucleic acid molecules are summarized in Table 8.

Selected nucleic acid molecules identified by screening for the expression of proteins recognized by Mozart II (immune) antiserum were subcloned and sequenced as described in Example 2.

Sequence analysis of nucleic acid molecules selected for expression of proteins recognized by Mozart II (immune) antiserum:

The nucleic acid molecules isolated using Mozart II (immune) serum were sequenced as described above. BLASTn and BLASTp homology searches were performed on these sequences using the NCBI GenBankTM non-redundant (nr)

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nucleotide (n) and amino acid (p) databases, and the dbEST (est) database as described above. The results of these searches are summarized in Table 3. Nucleic acid molecule M2A3 was sequenced again and some changes found between the first and second sequence. The resequenced nucleic acid molecule is referred to herein as M2A3-a. In addition, nucleic acid molecule M2A18 was sequenced again and some changes found between the first and second sequence. The resequenced nucleic acid molecule is referred to herein as M2A18-a.

The sequence data described above were used to design unique primers specific to each nucleic acid molecule of the present invention. These primer sequences are listed in Table 4. The unique primers listed in Table 4 were used in reverse transcriptase-polymerase chain reaction (RT-PCR) assays to assess the expression of the particular nucleic acid sequence in ICG, bradyzoites and tachyzoites. The results of these assays are summarized in Table 8.

Recombinant nucleic acid molecules, protein molecules and cells including sequences encoding *T. gondii* antigenic proteins and sequences from the vector pTrcHisB:

Recombinant nucleic acid molecules including sequences encoding T. gondii antigenic proteins and sequences from the vector pTrcHisB were produced as described in Example 2. In brief, T. gondii DNA fragments in λ gt11 were PCR amplified from nucleic acid molecules herein designated 4CQA-11, 4CQA-19, 4CQA-21, 4CQA-22, 4CQA-24, 4CQA-25, 4CQA-26, 4CQA-27, 4CQA-29, Tg-41, Tg-45, and Tg-50 using the λ gt11 forward and reverse primers herein described. The resulting recombinant molecules were transformed into E. coli to form recombinant cells. Immunoblot analysis of the recombinant cell lysates using a T7 tag monoclonal antibody (available from Novagen Inc., Madison, WI) directed against the fusion portion of the recombinant Toxoplasma fusion protein was used to confirm the expression of the fusion proteins and to identify their size. The results of this immunoblot analysis are summarized in Table 8.

Example 7:

This Example discloses a method of isolation of *T. gondii* nucleic acid molecules encoding immunogenic *T. gondii* proteins recognized by cat immune sera enriched for

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antibodies to gametogenic stages (herein referred to as absorbed immune sera or serum). This Example further discloses recombinant nucleic acid molecules, proteins and cells of the present invention.

Production of cat immune sera enriched for antibodies to gametogenic stages:

Sera from cats which were infected and then subsequently challenged with mouse brain-derived tissue cysts were tested for reactivity to extracts of infected cat gut material by Western blot analysis. Sera from one specific cat, designated Queen 2, demonstrated reactivity to particular ICG sections in which the presence of *T. gondii* had been shown by immunofluorescence assay. Queen 2 was originally infected with 100 mouse brain-derived tissue cysts, did not shed oocysts, and seroconverted to positive for tachyzoite antigens by day 39 post-infection. This sera was highly reactive to the asexual stage, tachyzoites. Therefore, to enhance the utility of this sera as a reagent for detection of gametogenic proteins, this sera was used in conjunction with a western blot of infected cat intestinal cell lysates to obtain a fraction enriched in antibody reactive to the gametogenic proteins. The enrichment of the Queen 2 sera (also referred to herein as Q2 sera) was performed as follows:

A 12% SDS-PAGE gel was prepared according to standard methods (Laemmli, 1970, *Nature 227*, 680-685). 1000 µg of solubilized ICG protein, prepared as described above, was loaded on 20 x 20 x 0.1 cm gel and run at 8V/cm for 5 hours. Toxoplasma tachyzoite (TZ) antigen, prepared from solubilized tachyzoites, was used as a control. Separated proteins were transferred to nitrocellulose according to standard procedures for western blotting. After transfer, the nitrocellulose filter was blocked with 4% (w/v) dry milk powder in PBS (pH 7.5), and incubated with a 1:200 dilution of immune cat (Queen 2) antiser m at room temperature for 5 hours with gentle shaking. The filter was then washed with PBS (pH 7.5). After washing, a 0.5 cm strip was cut off the end of the filter and incubated with a 1:1000 dilution of alkaline phosphatase labeled goat anti-cat IgG antibody at room temperature for 1 hour. The strip was stained with 5-bromo-4-chloro-3-indolylphosphate p-toluene salt/nitroblue tetrazolium chloride substrates (BCIP/NBT)(available from Gibco/BRL). The areas of the gel that stained with BCIP/NBT substrates represented ICG protein bands which were recognized by IgG antibodies in immune cat serum

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The regions of interest that were visualized on the BCIP/NBT-stained end strip were cut from the remainder of the filter, and the bound antibody eluted with 0.1 M glycine (pH 2.8), 1 mM EDTA at room temperature for 10 minutes. The antibody in glycine was neutralized with 10 mM Tris (pH 9.0), 0.02% NaN₃ was added, and the solution was stored at 4° C. The purified antibody was analyzed by Western blot of ICG to monitor successful recovery of the eluted antibody, verifying recovery of antibody that reacted with the appropriate molecular weight region of the ICG western blot. This antibody preparation is referred to herein as absorbed immune serum or sera.

The absorbed immune serum was used to isolate nucleic acid molecules herein designated Q2-4, Q2-9, Q2-10, and Q2-11 as follows: *E. coli* Y1090 was infected with approximately 3.2X10⁵ PFU and then evenly spread on 8 LB-amp agarose culture plates. The rest of the screening procedure was as described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:200 dilution, and the secondary antibody was a 1:1000 dilution of AP-conjugated goat anti-cat IgG. Of the 3.2 x 10⁵ plaques screened in this manner, 4 nucleic acid molecules capable of expressing proteins recognized by absorbed immune serum were plaque purified. The results of assays to characterize these nucleic acid molecules are summarized in Table 9.

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Table 9

Nucleic Acid Molecules Selected with Absorbed Immune Sera

	_		_	_	_	7	_	_		_		_
					2				•		•	
		2410		U	SEN CIN			+		+		+
		DDVAC		N VIVO				2		2		2
		Qa		IN VITRO IN VIVO				2	2	2	2	2
		2		7520			4	-			+	
	EYDDECCION	EAL RESS	DITUMO	2112	_		2			9	2	
		1	BZ			1	+7	-	٠		-	1
	DETECTION		71			-	-	4	-	+		+
	DETE	100	9					,				•
		2	?			5+		+		+		•
ORIGINAL	SECTION DESIGNATION				, , ,	UZ-4	0.00	GZ-9	02 40	01-27	02 11	42-11
0.00	SEG ID NO				٥	S	13	2	15	2	12	

Table 9 Legend: See Legend for Table 2.

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Selected nucleic acid molecules identified by screening for the expression of proteins recognized by absorbed immune serum were subcloned and sequenced as described in Example 2.

Sequence analysis of nucleic acid molecules selected for expression of proteins recognized by absorbed immune serum:

The nucleic acid molecules selected for expression of proteins recognized by absorbed immune serum were sequenced as described above. BLASTn and BLASTp homology searches were performed on these sequences using the NCBI GenBank™ non-redundant (nr) nucleotide (n) and amino acid (p) databases, and the dbEST (est) database as described above. The results of these searches are summarized in Table 3. Nucleic acid molecule Q2-9 was sequenced again and some changes found between the first and second sequence. The resequenced nucleic acid molecule is referred to herein as O2-9-a.

The sequence data described above were used to design unique primers specific to each nucleic acid molecule of the present invention. These primer sequences are listed in Table 4. The unique primers listed in Table 4 were used in reverse transcriptase-polymerase chain reaction (RT-PCR) assays to assess the expression of the particular nucleic acid sequence in ICG, bradyzoites and tachyzoites. The results of these assays are summarized in Table 9.

Recombinant nucleic acid molecules, protein molecules and cells including sequences encoding *T. gondii* antigenic proteins and sequences from the vector Prcro/T2ori/RSET-B:

Recombinant molecules containing *T. gondii* nucleic acid molecule operatively linked to lambda phage transcriptional control sequences and to a fusion sequence encoding a poly-histidine segment in the vector Prcro/T2ori/RSET-B, were produced essentially as described above, resulting in the production of recombinant molecule. The resulting recombinant molecules were transformed into *E. coli* to form recombinant cells using standard techniques as disclosed in Sambrook et al., *ibid*. Immunoblot analysis of expression of immunogenic *T. gondii* proteins by these recombinant cells is summarized in Table 9.

Example 8:

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This Example describes the construction of several cDNA expression libraries of the present invention.

A T. gondii tachyzoite cDNA expression library, a T. gondii infected cat gut (ICG) cDNA library (constructed from seven day post infection infected cat gut material, which is a mix of both cat intestinal cDNA and T. gondii gametogenic cDNA), and an uninfected cat gut (UCG) cDNA expression library were from total RNAs as follows:

Isolation of Total RNA From Tachyzoites: Total RNA from tachyzoites was prepared using Tri-ReagentTM (available from Molecular Research Center, Inc,

Cincinnati, Ohio) according to the manufacturer's directions. Briefly, 4 X 109 tachyzoites were resuspended in 6 ml of TriReagent with a syringe and 18 gauge needle. Successive triturations were made with 20 gauge and 22 gauge needles. A volume of CHCl₃ equal to one-fifth the original volume of TriReagent[®] was added and the mixtures were shaken for 15 seconds. The aqueous and organic phases were then separated by centrifugation. Total RNA was recovered from the aqueous phase by precipitation in isopropanol.

PolyA⁺ RNA was isolated from total RNA using Pharmacia mRNA purification kit (available from Pharmacia Biotech Inc., Piscataway, NJ).

Isolation of Total RNA from Other Sources: The method of isolation of total

RNA from various tissues was the same for all tissues. The only variable was the starting material. For example, to obtain RNA from infected cat gut (ICG) or uninfected cat gut (UCG), the epithelial layer of a fifteen square centimeter section of gut was scraped into 6 ml of Tri-Reagent and processed as described above. RNA from mouse was obtained from 1 gm of mouse brain and treated with Tri-Reagent as described above. RNA from bradyzoites was obtained from 7,000 tissue cysts propagated in mouse brain, obtained as described, and treated with Tri-Reagent as described above.

PolyA+ mRNA was isolated from total RNA using Pharmacia mRNA purification kit (available from Pharmacia Biotech Inc., Piscataway, NJ).

Preparation of λ cDNA libraries:

The ZAP-cDNA® synthesis kit (available from Stratagene) was used according to manufacturer's instructions to synthesize cDNA. Briefly, 5 or 10 µg of PolyA⁺ mRNA (prepared as described above) was reverse transcribed using Superscript® reverse transcriptase and 0.6 mM dGTP, dATP, dTTP, and 0.3 mM 5-methyl dCTP and 1.4 µg of oligo dT linker primer supplied with the ZAP-cDNA® Synthesis Kit. The second strand was made by digesting the RNA template with RNaseH and priming second strand synthesis with DNA polymerase I. The cDNA was then ligated into the Uni-ZAP® XR lambda insertion vector (available from Stratagene), packaged and amplified to produce tachyzoite and ICG cDNA libraries.

 $5~\mu g$ of polyA+ RNA was used to prepare the ICG cDNA library, and $10~\mu g$ of polyA+ RNA was used to prepare the tachyzoite cDNA library. For each library, 100~ng of double stranded cDNA was ligated and packaged and gave approximately $1.5~X~10^6$ unique nucleic acid molecules. The average size of the cloned inserts was 1.9~Kb in the tachyzoite cDNA library, and 2.1~Kb in the ICG cDNA library.

Example 9:

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This Example describes the construction and identification of cDNA sequences encoding near full-length *T. gondii* nucleic acid molecules encoding immunogenic *T. gondii* proteins.

Two of the molecular libraries described above were used to isolate near full-length *T. gondii* nucleic acid molecules encoding immunogenic *T. gondii* proteins: the tachyzoite cDNA library and the ICG cDNA library constructed from seven day post infection infected cat gut material.

The general approach to isolating nucleic acid sequences representing full length, or near full-length cDNA sequences was as follows: First, the MacVector DNA analysis program was used to design DNA primers for each of the Toxoplasma sequences cloned in an expression vector as herein described. These primers were then used in a PCR reaction in which the template was either of the Toxoplasma cDNA libraries herein described. The presence of a positive band on an agarose gel following PCR was diagnostic of the presence in the cDNA library of a nucleic acid molecule with homology to the primers. A near full-length cDNA molecule having sequence homology with the

genomic DNA sequence designated Q2-4 was obtained by a direct hybridization screen of the libraries using radiolabeled clone-specific PCR fragments as templates. The isolation of one of these near full-length sequences is herein described in detail as representative of the methods used to isolate all of the near full-length sequences identified by this strategy.

A cDNA sequence representing a near full-length gene having homology to a nucleic acid sequence herein designated Q2-4 (isolated from the Toxoplasma genomic DNA library) was isolated from the infected cat gut (ICG) cDNA library by hybridization screening as follows: E. coli Y1090 was infected with approximately 1X106 PFU of the Toxoplasma ICG cDNA library and then plated at a density of about 10 50,000 plaques per 150 mm agar plate. The resulting plaques were transferred to nitrocellulose filters. The filters were then soaked in denaturing solution (1.5 M NaCl, 0.5 M NaOH) for two minutes, neutralization solution (1.5 M NaCl, 0.5 M Tris, pH 8) for five minutes, and then rinsed several times in 2 X SSC (150 mM NaCl, 15 mM Na citrate, pH 7). The DNA bound to the filters was crosslinked using a Stratalinker® UV crosslinker (available from Stratagene) according to the manufacturer's directions.

A radioactive hybridization probe was made by incorporating 32P into clonespecific template DNA using a Prime-It® II random primer labeling kit (available from Stratagene) following the manufacturers directions. The template was a PCR fragment generated by using two primers specific for Q2-4. For each 100 µl reaction, 30 ng of Toxoplasma genomic DNA was PCR amplified using 200 mM of each dCTP, dGTP, dTTP, dATP, 200 nM of each specific primer, 2.5 mM MgCl₂, 20 mM Tris pH 8.4, 50 mM KCl, and 2.5 units Taq DNA polymerase (available from The Perkin Elmer Corp.) for thirty-five cycles in a Perkin-Elmer Gene Amp PCR System (available from The Perkin Elmer Corp.).

The nitrocellulose filters containing crosslinked DNA were hybridized in 2 X PIPES buffer (10 mM piperazine-N, N'-bis[2-ethanesulfonic acid] (pH 6.5), 400 mM NaCl), 50% formamide, 0.5% SDS, 100 mg/ml denatured salmon sperm DNA and 10^7 cpm/ml of the radioactive hybridization probe. The filters were incubated with this hybridization solution overnight at 42° C. The next day the filters were washed in $0.1~\mathrm{X}$

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SSC, 0.1 % SDS and then exposed to X-ray film (available from Kodak, Rochester, NY) in order to visualize positive plaques.

Plaques in the area corresponding to the positive signals were picked into SM buffer (50 mM Tris, pH 7.5, 100 mM NaCl, 8 mM MgSO₄, and 0.01% gelatin) and the phage replated at a lower density. The same screening procedure was repeated three or four times until a pure plaque corresponding to a full length cDNA nucleic acid sequence representing Q2-4 was isolated.

After plaque purification, the nucleic acid molecules were mapped and the areas of interest sequenced using primers specific to the original clone, long fragment PCR, and cycle sequencing of the large fragments.

Example 10:

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This Example describes the expression in a eucaryotic cell of nucleic acid molecules encoding immunogenic *T. gondii* proteins, and DNA vaccination with nucleic acid molecules encoding immunogenic *T. gondii* proteins.

Cloning into a eucaryotic expression vector(pDVacI):

Inserts from eight clones (OC-2, OC-13, OC-14, OC-22, Tg-11, Tg-15, Tg-50, 4CQA-11) were ligated into the pDVacI expression vector. This vector contained a eucaryotic promoter from cytomegalovirus (CMV), followed by the start codon and signal sequence for a mouse kappa immunoglobulin gene. An *EcoR* I site was inserted in frame downstream to the signal sequence. This allowed the insertion of *EcoR* I fragments directly from the original lambda phage. The nucleic acid molecules produced by insertion of nucleic acid molecules encoding immunogenic *T gondii* proteins into pDVacI are referred to herein as pDVacI:Toxoplasma nucleic acid molecules. If the *EcoR* I inserts represent nucleic acid sequence that is entirely open reading frame, then the protein product expressed from these inserts may be in frame with a C-terminal fusion consisting of both a poly histidine track and amino acid sequence representing an epitope from the human *myc* gene as a reporter sequence. The N-terminal fusion adds 49 amino acids, or about 5.4 kD to the protein encoded by the *T gondii* nucleic acid molecule, and the C-terminal fusion adds 38 amino acids. or about 4.2 kD, to the fusion protein.

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Expression in vitro:

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Direct sequencing of the inserts in each plasmid confirmed the production of eight different pDVacI:Toxoplasma nucleic acid molecules. DNA from these molecules was then tested for eukaryotic expression of antigenic *T. gondii* proteins by transfecting BHK cells *in vitro* with DNA isolated from the pDVacI:Toxoplasma nucleic acid molecules. Analysis of the eukaryotic expression products of the pDVacI:Toxoplasma nucleic acid molecules was done by western blot on cell lysates and on supernatants from the transformed BHK cells. Either a monoclonal reactive with the *myc* epitope or antibody specific to each clone was used as the primary antibody. Seven out of the eight plasmid constructs expressed a protein *in vitro*. See Table 10.

Table 10

Analysis of Clones in Eucaryotic Expression Vector and DNA Vaccination

o do lo	Size (KD) Expressed in	EU / ug DNA*	Expressic Pellet	Expression <i>in vitro</i> Pellet Super	Sero- conversion (# of Mice)**
		6			(22)
0C-2	40	0.3 / 0.4	+	+	5/2/2
OC-13	38	0 / 0.23	+	+	0/0/4
OC-14	32	7.7 / 3.8	•	ı	**
OC-22	40	0.5 / 0.44	+	+	4/5/5
Tg-41	33	23 / 1.8	+	+	0/1/5
Tg-45	26	0/0	+	+	3/5/5
Tg-50	55	4.0 / 4.0	+	+	5/5/5
4cqa-11	25	0.95 / 5.3	+	+	0/0/0

Table 10 legend:

(*) The first and second numbers represent the endotoxin units (EU)/ug of DNA of mice that sero-converted at the 4, 7, and 9 week bleeds, respectively, out of the group for the first and second immunizations respectively. (**) The numbers represent the # of five that were injected. (***) Antigen for Nt4 protein was not available to analyze for these sera samples. 5

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Expression in vivo:

100 ug of each pDVacI:Toxoplasma nucleic acid molecule was injected intradermally into five mice. The administrations were at day zero and week five; bleeds were collected at weeks four, seven and nine. The mouse sera were used to determine if the DNA vaccination with each clone elicited a serological response to the cloned fusion protein. This was measured by western blot analysis with the protein expressed in the BHK lysates. Six of the eight clones induced antibodies in mice by week nine, see Table 10.

Reactivity of antibody raised against recombinant OC-1 protein:

Purified recombinant protein expressed by an expression vector containing the nucleic acid sequence referred to as OC-1 (SEQ ID NO:70) was used to immunize mice and rabbits by methods well known in the art. The animals were blcd, and scrum collected used in immunofluorescence assays against infected and uninfected cat gut tissue. The results of these assays showed that antibody raised, in mice and rabbits, to recombinant OC-1 protein bound to most of the enteroepithelial stages in the infected cat gut. The antiserum did not react with uninfected cat gut.

Example 11:

This example describes the construction of a Toxoplasma gondii EMBL3 genomic library from tachyzoites grown in tissue culture. This Example further describes isolation of near full-length nucleic acid molecules encoding stage specific T. gondii antigenic proteins.

An EMBL3 library of Toxoplasma genomic DNA was constructed using standard molecular cloning methods, well known to those skilled in the art of cloning (see, for example, Sambrook, et al., ibid.). In brief, Toxoplasma genomic DNA. prepared from tachyzoites as herein described, was partially digested with Sau3A I, using a series of different ratios of units of enzyme to µg of DNA. Digestions were incubated at 37°C for one hour. Ratios of 0.06, 0.03, and 0.015 units of enzyme per µg of DNA produced high molecular weight DNA fragments which were then run on a preparative agarose gel. The fraction of the gel corresponding to DNA in a size range of between 15 and 20 Kb was excised. The DNA fragments were extracted from the gel, and the amount of extracted DNA quantitated. The EMBL3 library was then constructed

using this DNA and the Lambda EMBL3/BamH I Vector Kit (available from Stratagene). The manufacturer's instructions were followed for all cloning steps, and the resulting ligated DNA was packaged using the Gigapack[®] II XL Packaging Extract (available from Stratagene). Packaging and amplification followed the manufacturer's specifications. The resulting library is referred to herein as the EMBL3:Toxoplasma genomic library.

The EMBL3:Toxoplasma genomic library was plated at a density of 50,000 plaques per 150 mM agar plate and the plaques transferred to a nitrocellulose filter. The filters were soaked in denaturing solution (1.5 M NaCl, 0.5 M NaOH) for two minutes, neutralization solution (1.5 M NaCl, 0.5 M Tris, pH 8) for five minutes, rinsed several times in 2 X SSC (150 mM NaCl, 15 mM Na citrate, pH 7), and the DNA crosslinked using a Stratalinker[®] UV crosslinker (available from Stratagene) according to the manufacturer's instructions.

The EMBL3:Toxoplasma genomic library was screened with probes made from PCR amplified nucleic acid molecules isolated by immunoscreening the λ gt11:Toxoplasma genomic library. The primers used to generate these probes were derived using the MacVector Sequence Analysis program and the sequences of nucleic acid molecules encoding *T. gondii* antigenic proteins isolated from the λ gt11:Toxoplasma genomic library.

The filters were hybridized in 2 X PIPES buffer (10 mM piperazine-N, N'-bis[2-ethanesulfonic acid] (pH 6.5), 400 mM NaCl), 50% formamide, 0.5% SDS, 100 μg/ml denatured salmon sperm DNA (available from Sigma) and 10⁷ cpm/ml of radioactive hybridization probe. The filters were hybridized overnight at 42°C. The next day the filters were washed in 0.1 X SSC, 0.1 % SDS, and then exposed to X-ray film (Kodak).

Plaques in the area corresponding to the positive signals were picked into SM buffer (50 mM Tris, pH 7.5, 100 mM NaCl, 8 mM MgSO₄, and 0.01% gelatin) and the phage replated at a lower density. The same screening procedure was repeated three or four times until a pure plaque hybridizing with a nucleic acid molecule isolated by immunoscreening the λ gt11:Toxoplasma genomic library was isolated. After plaque purification, the nucleic acid molecules were mapped and the areas of interest sequenced

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using primers specific to the original clone, long fragment PCR, and cycle sequencing of the large fragments.

Long fragment PCR was done with a Perkin-Elmer XL PCR kit (available from The Perkin-Elmer Corp., Foster City, CA) as follows: A 100 µl reaction was separated into two layers with a wax bead so one would have a hot-start reaction. The lower layer contained 1 X XL PCR buffer supplied with the kit, 40 pM each of the forward and reverse primers, SC1011 and SC1002, (supplied by the manufacturer with the XL PCR kit, 2.5 mM each dNTP, 1.1 mM Mg(OAc)₂. The upper layer contained 1 X XL buffer, 4 units of rTth DNA polymerase (available from The Perkin-Elmer Corp.) and about 5 ng of the plaque purified EMBL3:Toxoplasma genomic DNA as the template. The reaction was done in a Hybaid thermocycler (available from Hybaid Ltd., Middlesex, UK), and the reaction products were resolved on a 0.6% agarose gel.

Example 12:

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This Example describes the detection of T. gondii oocysts in cat feces by PCR amplification of nucleic acid sequences homologous to nucleic acid sequences encoding immunogenic T. gondii proteins of the present invention. Specifically, this example describes a rapid PCR dipstick method for the detection of oocysts in feces.

Naive cats were infected per os by 1000 mouse-brain derived tissue cysts of T. gondii strain C at day zero. Feces from each animal were collected, if available, on a daily basis starting at day zero and each day for 19 days post infection (PI). A portion of the feces was treated by the standard sugar floatation method (Dubey, J.P., Swan, G. V., and Frenkel, J. K. 1972, Journal of Parasitology. 58: 1005-1006) and the oocysts visualized using a microscope and counted on a haemacytometer. A portion of each feces was also suspended in PBS, vortexed and a small sample obtained by dipping an IsoCodeJ ™ dipstick (available from Schleicher & Schuell, Keene, NH) into the fecal solution. The dipstick was allowed to air dry and then washed in 500 ul of distilled water by vortexing the stick end and water in a tube for 10 seconds. Material adhering to the filter was then eluted in 50 µl of fresh distilled water by heating to 95° C for 30 minutes. The remaining supernatant was then used for standard hot start PCR, according to methods well known in the art, using primers representing DNA sequences from nucleic acid molecules encoding T. gondii antigenic proteins. The results of an

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experiment in which primers derived from nucleic acid molecule OC-2 were used are shown in Table 11. The results of this experiment demonstrated that the PCR detection method was at least as sensitive at detecting oocysts in fecal matter as the conventional floatation method.

Table 11

PCR Analysis of Cat Feces

	PCR Dipstick Oc2				•	• 4	- +	- 4	-	+	- +	· +		,	,	ı					ı
#3515.1	Oocysts/gm Float Di	C	o c	o c	o c	1X10e6	5X10e6	1X10e6		2X10e5	7X10e4	0	0	0	0	C	0	0	0	0	0
	Day PI	O	· -	٠	l m	4	. 2	9	7	- ∞	တ	10	7	12	13	4	15	16	17	18	19
	PCR Dipstick Oc2		•	,	ı		+	+	+	+	+	+	+	+	•	•	•			,	4
#3512-1	K <u>Oocysts/gm</u> F Float Dig	0	0	0	0		1X10e4	3X10e5	1X10e6	1X10e6	1×10e6	1X10e5	1X10e5	0	0	0	0			0	0
	Day PI	0	-	2	က	4	S	9	7	æ	6	10	7	12	13	4	15	16	17	18	19
	PCR Dipstick Oc2	•	•	ı	•	ı	•		ı		•	ı	•				•				•
#3528-U	V Oocysts/gm I	0	0	0	0	0	0		0		0	0	0				0				0
	Day PI	0	_	7	က	4	2	9	7	æ	თ ·	9	-	15	. .	1 4	15	16	17	<u>æ</u>	1 0

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A series of additional experiments was performed in order to investigate further the PCR dipstick method for the detection of oocyts in feces. In this set of experiments, the following methods were used to produce T. gondii infected cats, and to detect oocysts in the feces of the infected cats. T. gondii C-strain tissue cysts were obtained by orally infecting 6-8 week old Swiss Webster mice with a sub-lethal dose of mouse brain derived tissue cysts. At six weeks post infection, the animals were euthanized with CO, and the brains were removed and placed in 30% Dextran in HBSS (Gibco/BRL). The brains were then homogenized with a Tissuemizer (Tekmar Co., Cincinnati OH) and centrifuged at 5,000 x g's for 10 min at 4°C. The pellet was resuspended in HBSS and the tissue cysts were counted. The tissue cysts were diluted with PBS to the appropriate concentration for oral administration to cats at the back of the throat using a 1ml syringe. A total of twenty cats was used in this study: seventeen were experimentally infected with 1000 tissue cysts and three were used as uninfected controls. All cats were housed in separate cages and feces were collected at the day of infection and daily for the next 21 days. On average there were approximately twelve samples per cat. The fecal samples were stored at 4°C until tested, which was within two weeks of collection.

Conventional quantification of oocysts in feces was based on the sugar flotation method of Dubey and Beattie, 1988, and is described in full as follows. Each fecal sample was weighed and then 2 grams of feces were mixed with 15 ml of sugar solution (53 gm sugar, 100 ml of water). Following solubilization with a tongue depressor, the mixture was passed through two layers of gauze. The filtrate was poured into a 15 ml conical tube and centrifuged at 1,200 x g for 10 minutes. The top 3 ml of the sample was added to 13 ml of sugar solution and centrifuged as above. The top 3 ml of the second flotation was added to 13 ml of water and centrifuged at 1,200 x g for 10 minutes. The resulting oocyst pellet was resuspended in 1ml of water and the oocysts counted using a hemacytometer. Alternatively, the entire fecal sample was solubilized in PBS by adding five ml of PBS per gram of the pre-weighed feces in a 250 ml plastic beaker. After one hour at room temperature, a tongue depressor was used to thoroughly suspend the feces. Five ml of the fecal slurry was added to a 15 ml tube containing 5 ml of 2X sugar solution and inverted several times. The tube was then centrifuged at 1,200

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x g for 10 minutes. The top 3 ml of the sample was subjected to a second sugar flotation, resuspended, and counted as described above.

Analysis of the fecal samples by the PCR dipstick method was performed as follows. One ml aliquots were taken, prior to further processing for floatation, from each of the initial fecal slurries described above. Samples were collected directly onto dipsticks, either by spotting 10 ul onto each dipstick filter or by directly dipping the dipstick into the fecal slurry. The filters were then dried at room temperature and the filter portion of the dipstick was cut off into a sterile 1.5 ml centrifuge tube. The filter was washed with 500 ul of sterile distilled water by vortexing for 8 seconds. The wash was removed and 50 ul of sterile water was added to the tube and adherent oocyst DNA eluted by heating at 95 °C for 1 hour. The filter was removed with a sterile tip and the sample stored (also referred to as the dipstick eluate) at -20 °C.

Primers specific to two T. gondii genes, B1 and OC-2, were used in the amplification reactions. The primers for the B1 gene (Burg, et al., 1989, Journal of Clinical Microbiology, 27: 1787-1792) were B1 forward (5'-GGA ACT GCA TCC GTT CAT GAG-3', herein referred to as SEQ ID NO:332), B1 reverse (5'- TCT TAA AGC GTT CGT GGT C-3', herein referred to as SEQ ID NO:333), and a B1 internal primer (5'-GGC GAC CAA TCT GCG AAT ACA CC-3', herein referred to as SEQ ID NO:334). The T. gondii OC-2 was isolated as herein described. The OC-2-derived primers were OC-2 forward (5'-GCA TCC TTG GAG ACA GAG CTT GAG-3', herein referred to as SEQ ID NO:335), OC-2 reverse (5'-GGG TTC TCT CGC TCA TCT TTC-3', herein referred to as SEQ ID NO:336), and an OC-2 internal primer (5'-AGT CAG AAG CAG TCA AGG C-3' herein referred to as SEQ ID NO:337). The PCR mixture contained 1X PCR buffer (10 mM Tris-HCl₂, 1.5 mM MgCl₂, 50 mM KCl), 0.2 mM deoxynucleoside triphosphates (Perkin-Elmer Cetus Corp., Norwalk, CN), 0.8 uM of each primer, 0.5 U of Gold AmpliTaq™ DNA polymerase (Available from Perkin-Elmer Corp.), and 1 ul DNA template in a total volume of 25 ul. The reaction mixture was denatured at 95°C for 10 minutes, amplified for 42 cycles including a denaturation step at 95°C for 30 seconds, annealing at 58°C for 30 seconds, extension at 72°C for 40 seconds, and a final extension for 5 minutes at 75°C on an automated DNA thermal

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cycler (Model 9700, Perkin-Elmer, Foster City, CA). PCR products were analyzed by electrophoresis on a 1.5 % agarose gel, stained with ethidium bromide (0.5 ug/ml), and photographed on a UV transilluminator.

Following electrophoresis, the DNA products were denatured in 0.5 N NaOH and 1.5 M NaCl buffer for 30 minutes, transferred to a nylon membrane (Maximum Strength Nytran Plus, available from Schleicher & Schuell) overnight and cross-linked by exposure to UV light (UV Stratalinker 1800, available from Stratagene). The filters were incubated in prehybridization buffer (5 x SSC, 1X Denhardt's reagent, 0.2% SDS, 1 mg/ml sheared DNA) at 42°C for 2 hours and then in hybridization buffer (5 x SSC, 1X Denhardt's reagent, 0.2% SDS, 1 mg/ml sheared DNA) containing 5' γ-³²P labeled oligonucleotide probe at 42°C overnight. After overnight incubation, membranes were washed twice in 2X SSC, 0.1% SDS for 15 minutes at room temperature, and then washed twice in 0.2X SSC, 0.1 % SDS at 55°C for 1 hour. The filters were autoradiographed at -70°C with Kodak XRR film.

Ethidium bromide-stained agarose gel and Southern hybridization analysis of PCR amplified products from oocyst-seeded fecal samples was performed in order to determine whether the dipstick method described herein resulted in a reduction of inhibition of PCR amplification of *T. gondii*-specific DNA in fecal slurries as compared with fecal slurries alone. Two sets of solutions, PBS and PBS/Feces (1:4 gm/ml), were seeded with four concentrations of oocysts, 2 x 10⁶, 5 x 10⁵, 5 x 10⁴, and 5 x 10³. Using the dipstick technique described above, this resulted in an estimated maximum number of oocysts in the PCR amplification tube to be 400, 100, 10, and 1 as indicated for the PBS solution and for the PBS/Feces solution respectively. Southern hybridization was performed using the OC-2 gene internal primer as the probe. Southern hybridization results and the ethidium bromide stained gel demonstrated that inhibition of PCR amplification of the exogenously added DNA was dramatically reduced (as compared with fecal extract alone) in samples prepared as per the dipstick assay as described above.

Three different paper supports were tested for their ability to support the PCR

30 dipstick assay: IsoCodeJ™ Stix, S&S® #903™ (available from Schleicher and Schuell)

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and Nobuto Blood Filter Strips (available from Advantec, Pleasantville, CA). First, IsoCode JTM Stix were tested for the ability to bind oocysts. Oocysts were diluted into either PBS or a suspension of uninfected feces and PBS. The fecal dipstick procedure as described above was used to sample and elute DNA for PCR analysis. The concentration of oocysts per reaction was adjusted so that theoretical maximum could be 1, 10, 100, and 400 oocysts respectively. The amplification products were run on an agarose gel and stained as described above. According to this assay, oocysts diluted into PBS alone could be readily detected at 10 oocysts per ul of dipstick eluate with primers directed to the *T. gondii* OC-2 gene. In addition, oocysts in a suspension of feces and PBS could be detected when present at a concentration of between 10 and 100 oocysts per ul. This experiment demonstrates that the oocysts are bound to the IsoCode JTM Stix in the presence of feces, are eluted by heat, and following a wash and heat elution step are sufficiently free from inhibitors to be detected by PCR amplification.

Under these conditions, detecting 10 oocysts per ul of eluate from the IsoCodeJ™ Stix is equivalent to detecting oocysts at a concentration of 2.5 x 10⁵ 15 oocysts/gram of feces. Several parameters were tested for their ability to increase the sensitivity of this test. First, two additional paper supports, S&S® #903™ and Nobuto Blood Filter Strips, were tested for both the ability to bind oocysts in the presence of solubilized feces, and the ability to support subsequent PCR detection of oocyst DNA. Each of these filter papers bound T. gondii oocysts, and subsequent PCR amplification 20 with OC-2 primers detected the presence of T. gondii DNA. However, the sensitivity of detection for each of these papers was somewhat less than the sensitivity of the assay when using IsoCodeJ Stix™. All three paper supports were also tested for binding of oocysts in the presence of feces over a range of pH from 4 to 9. The S&S® #903™ and Nobuto Blood Filter Strips were most effective at pH 7. Binding of oocysts to the 25 IsoCodeJ Stix™ was significantly increased at pH 9. All subsequent assays described below used IsoCodeJ Stix™ and pH 9 for binding of oocysts to dipsticks.

Another approach to increasing the sensitivity of the assay was to use primers from the B1 gene during the PCR amplification reaction. The B1 gene is a multicopy gene that is present at approximately 35 copies per T. gondii genome. Using a B1-

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specific primer resulted in a ten-fold increase in sensitivity, and produced an assay in which I oocyst/ul could routinely be detected. This level of sensitivity of the assay correlated with the ability to detect approximately I x 10⁴ oocysts/gram of feces.

The sensitivity and specificity of the PCR detection method was tested in

sexperimentally infected animals using flotation and visualization of oocysts as the standard for quantification of oocysts. SPF cats were infected with mouse brain-derived tissue cysts and feces were collected from the cats for twenty-one days. Each sample was analyzed by both direct visualization and the dipstick PCR technique. Following gel electrophoresis of the products from PCR amplification, the results were scored as

either positive or negative depending on the presence or absence of the correct genespecific PCR product. Table 12 shows the results of PCR detection using both the B1 and OC-2 DNA primers for each individual fecal sample. The positive and negative predicative values were 93.2% and 97.2% respectively using the B1 gene DNA primers and 80.2% and 95.8% respectively using the OC-2 DNA primers.

15 TABLE 12. Sensitivity, specificity and predicative values for the PCR detection of oocysts in experimentally infected cat feces.

Method	Total Samples +/-	f/n ^a	f/p ^b	Sensitivity %	Specificity %	Predictive Value % +/-
Microscopy	69/176	.0	0	100	100	100/100
PCR						
B1 Primers	64/171	5	5	94.7	96.7	93.2/97.2
OC-2 Primers	61/161	7	16	89.7	96.4	80.2/95.8

^a false negative

Example 13:

A PCR ELISA was developed for the detection and quantification of PCR amplification products from the PCR dipstick method. In general, digoxigenin-labeled amplified product produced by the PCR dipstick detection method were detected by hybridization to an internal biotinylated B1 gene primer bound to microtiter wells. The

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b false positive

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concentration of PCR labeled digoxigenin fragment was determined using an alkaline phosphatase-linked anti-digoxigenin antibody (available from Boehringer Mannheim Biochemica Gmbh). The alkaline phosphatase activity level was then determined using a standard ELISA reader. This quantitative PCR ELISA method detected oocysts at a lower limit of 1 x 10⁴ oocysts/gram when tested with uninfected cat feces seeded with known concentrations of T. gondii oocysts. The method is described in detail as follows.

PCR amplification using B1 gene-specific primers was performed on eluates from the fecal dipstick method herein described. Amplification products were labeled by incorporation of digoxigenin-11-dUTP (DIG-11-dUTP) present in the reaction mix at 2.5 uM. The concentration of dTTP in this reaction mix was reduced to 22.5 uM. The 10 resulting labeled fragment was detected using reagents from the PCR ELISA (DIG Detection) kit (available from Boehringer Mannheim Biochemica Gmbh, Mannheim, Germany). The procedure was as follows. Four ul of the primary amplification reaction product was added to 16 ul of denaturation buffer and incubated at room temperature for 10 minutes. This was mixed with 200 ul hybridization buffer that contained 20 pmol/ml of the biotinylated B1 gene probe. One-half of the hybridization reaction mixture was transferred to a well in a streptavidin-coated microtiter plate and incubated at 50°C for 3 hours with shaking. The plate was washed with washing buffer five times at room temperature and incubated with 100 ul of anti-digoxigenin Fab conjugated with peroxidase at 37°C for 45 minutes. Following five washes, 100 ul of ABTS substrate solution (available from Boehringer Mannheim Biochemica) was added to each well and the color was developed at room temperature for 45 minutes. The optical densities (OD) at 405 nm were read in a spectrophotometer (SpectraMAX 250, available from Molecular Devices Inc., Sunnyvale, CA) and analyzed with Soft Max Pro™ software (available from Molecular Devices Inc.).

Quantification of oocysts in feces by the PCR ELISA technique was compared with quantification by the microscopic analysis. Individual feces from six different cats were collected (as available) at various days post infection. Oocysts were then quantified for each sample by two separate techniques, microscopy and PCR ELISA.

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The results from each of these two methods were in good agreement. Standard regression analysis produced a correlation coefficient of 0.91.

Example 14:

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This example describes the detection of Cryptosporidium parvum oocysts and Giardia lamblia cysts in feces using the PCR dipstick detection method described above. Oocysts and cysts from C. parvum and G. lamblia respectively were detected by the dipstick PCR detection method, thereby demonstrating the usefulness of this method for the detection of cysts or oocysts from unrelated species.

Feline fecal samples from SPF cats were seeded with either *C. parvum* oocysts or *G. lamblia* cysts and used in the PCR detection method described herein. The primers used to detect *C. parvum* were specific for the *C. parvum AWA* gene, while the primers used to detect *G. lamblia* were specific for the *G. lamblia ABB* gene (Rochelle, et al., 1997, *Applied and Environmental Microbiology* 63:106-114).

In order to demonstrate binding of *C. parvum* oocysts to a dipstick in the presence of feline fecal slurry, aliquots of feline fecal slurry (1:4, mg/ml) were seeded with between 5X 10² and 5X 10⁶ *C. parvum* oocysts/ml. These samples were then tested for binding of the oocysts and subsequent PCR analysis according to the PCR detection methods described herein. The primers used in the PCR amplification were specific for the *C. parvum AWA* gene. The PCR amplified products were run on and agarose gel and stained with ethidium bromide. The *C. parvum*-specific primer primed amplification of a DNA product of the predicted mobility, in an oocyst concentration-dependent manner, from the dipstick eluate as described above. The results of this experiment demonstrated that *C. parvum* oocysts bound to a dipstick in the presence of feline fecal slurry, and that about 5X 10² *C. parvum* oocysts/ml were detectable by the PCR detection method after binding to the dipstick under these conditions. Because 5X 10² oocysts/ml was the lowest concentration tested, and the products were easily observable, the concentration of cysts detectable by this method is likely to be lower than 5X 10² oocysts/ml.

In order to demonstrate binding of *Giardia* cysts to a dipstick in the presence of feline fecal slurry, aliquots of feline fecal slurry (1:4, mg/ml) were seeded with between 5X 10² and 5X 10⁵ G. lamblia cysts/ml. These samples were then tested for binding of

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the cysts and subsequent PCR analysis according to the PCR detection methods described herein. The primers used in PCR amplification were specific for the G. lamblia ABB gene. The PCR amplified products were run on and agarose gel and stained with ethidium bromide. The G. lamblia-specific primer primed amplification of a DNA product of the predicted mobility, in a cyst concentration-dependent manner, from the dipstick eluate as described above. The results of this experiment demonstrated that G. lamblia cysts bound to a dipstick in the presence of feline fecal slurry, and that about 5X 10² G. lamblia cysts/ml were detectable by the PCR detection method after binding to the dipstick under these conditions. Because 5X 10² cysts/ml was the lowest concentration tested, and the products were easily observable, the concentration of cysts detectable by this method is likely to be lower than 5X 10² cysts/ml. Example 15:

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This Example discloses a method of isolation of T. gondii nucleic acid molecules encoding immunogenic T. gondii proteins recognized by intestinal secretions from infected cats. This Example further discloses recombinant nucleic acid molecules and proteins of the present invention.

The production of intestinal secretions and from infected cats and the use of these secretions for screening for nucleic acid molecules encoding immunogenic T. gondii proteins are described herein in Example 6. Intestinal secretions collected from a single cat that had been previously infected with T. gondii were pooled and preabsorbed to remove antibodies directed against UCG and E. coli. The pooled, preabsorbed intestinal secretions are also referred to herein as MGIS antiserum. MGIS antiserum was used to immune screen an ICG cDNA library in order to identify and isolate nucleic acid molecules encoding immunogenic T. gondii proteins recognized by intestinal secretions from infected cats. Six nucleic acid molecules encoding immunogenic T. gondii proteins recognized by intestinal secretions from infected cats were identified and isolated using the following methods. These six nucleic acid molecules are referred to herein as MGIS4-2 (also herein referred to as SEQ ID NO:282 and SEQ ID NO:284, representing the coding strand and its reverse complement, respectively), MGIS4-4 (also herein referred to as SEQ ID NO:292 and SEQ ID NO:294), MGIS4-8 (also herein

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referred to as SEQ ID NO:306 and SEQ ID NO:308), MGIS6-5 (also herein referred to as SEQ ID NO:311 and SEQ ID NO:313), MGIS6-2 (also herein referred to as SEQ ID NO:326 and SEQ ID NO:328), and MGIS1-3 (also herein referred to as SEQ ID NO:329 and SEQ ID NO:331).

Absorption of MGIS Antibody

MGIS antiserum was collected, as previously described, from the cat intestine on weeks 6, 10, and 13 after infection, and on weeks 0, 1, 2, 3, 4, and 5 after challenge. Both pools of antisera were combined and used to screen the cDNA library, and are herein referred to as MGIS antiserum.

To remove anti-cat intestinal and anti-*E.coli* tissue reactive antibodies, the MGIS pools were absorbed to nitrocellulose (NC) filters coated with either cat intestinal proteins or *E.coli* proteins. Cat intestinal proteins used to coat the nitrocellulose filters were generated as follow. The epithelial layer of uninfected cat intestine was scraped on dry ice and the cells subsequently passed through several different gauge needles (No. 18, 21, and 23) 10 times each. The sample was frozen and thawed 3 times, and then sonicated on ice for 10 minutes. The protein extract was diluted to 400ug/ml in PBS and immersed with the nitrocellulose at room temperature for 1 hour, and was then blocked with 4% milk in PBS for 30 minutes. Similarly, XL-1 blue *E.coli* cells were resuspended in PBS and bacterial protein extracts prepared similar to the cat intestinal proteins. The bacterial extract was diluted to a final concentration of 2.3 mg/ml in PBS and bound to the filter in a manner similar as the cat intestinal extract.

MGIS antiserum was diluted 1:20 with 4% milk in PBS and absorbed sequentially to both the cat intestinal and bacterial protein coated filters at room temperature for 1 hour. To demonstrate that all UCG and *E. coli*-reactive antibody had been removed from the MGIS antiserum preparation, the MGIS antiserum subjected to Western blot analysis which showed that the absorbed antibody had no reactivity to either the cat intestinal proteins or to the bacterial extract.

Immune Screening of T. gondii cDNA Phage Library

The ICG cDNA library was constructed from infected cat intestinal mRNA, and the cDNA product cloned into the *EcoRI/XhoI* sites of the Uni-Zap XR vector.

Toxoplasma-specific nucleic acid molecules represented approximately 10% of the library. The ICG cDNA phage library was plated to approximately 2-5x10^{e4-5} pfu per 135 mm plates with XL-1Blue MRF' cells (available from Stratagene). Ten plates were treated in the following manner after the phage were pinhead in size. Nitrocellulose filters that had been previously treated with IPTG were overlaid on top of the phage and incubated at 37°C for 5 hours. The filters were marked, washed with TBS, pH 8.0, blocked with 4% milk in TBS, and incubated with MGIS antiserum at room temperature overnight. After washing three times with TBS, horse-radish peroxidase (HRP)-labeled goat anti-cat IgA antibody (Bethyl Lab. Inc.) was diluted 1:350, and incubated with the filters at room temperature for 2 hours. The color indicator was developed with 4-10 chloro-1-naphthol substrate and H₂O₂. Forty-one positive clones were selected for further screening.

Hybridization Screening and Clone Purification

Selected clones were replated on NZYM plates, and forty-eight individual plaques randomly picked and resuspended in 100 ul of SM buffer. Insert DNAs were 15 PCR amplified in a final volume of 12.5 ul containing 1 ul of template DNA, 50mM KCL, 10mM Tris-HCL (pH 8.3), 2mM MgCl₂, 0.2mM each dNTP, 0.2mM each of T3 and T7 vector specific oligonucleotide primers, and 0.3 units of Taq polymerase. Amplification was performed by 1 cycle of 95° C for 3 min., 35 cycles of 95° C for 30 sec., 50° C for 30 sec., and 72° C for 2 min., followed by 75° C for 5 min. on a Perkin 20 Elmer 9600 thermocycler. The PCR amplified products were analyzed on a 1% agarose TBE gel, and the DNA transferred to a nylon membrane.

A hundred nanograms of T. gondii genomic DNA was labeled using the Megaprime DNA labeling systems (available from Amersham International) and used as a probe to analyze the PCR amplified DNA fragments on the nylon membrane. The membrane was pre-hybridized in 5xSSPE (1x SSPE: 0.18M NaCl, 10mM NaH₂PO₄, and 1 mM EDTA pH 7.7), 0.5% SDS, 5x Denhardt's solution, and 0.1 mg/ml single stranded salmon sperm DNA at 65° C for 3 hours. Membranes were then hybridized overnight at 65° C, and then washed with 2xSSPE, 0.1% SDS at room temperature for 10 min., twice,

and 0.2xSSPE, 0.5% SDS at 65° C for 1 hour, twice. The membrane was exposed to 30

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film at -70° C overnight. Twenty-three clones were thus shown to contain T. gondii-specific DNA, with an insert size of 1-2 Kb in length.

Clone Identification by Phage Drop Test

Each of the twenty-three *T. gondii*-specific clones were rescreened to confirm reactivity with MGIS antiserum. Phage clones were diluted 1:10e7 from the SM buffer stock, and 3ul of this dilution (~5-50 phage) was spotted onto a NZYM/XL-1Blue MRF' agar plate, and incubated at 37° C for 5 hours. Afterwards, an IPTG pre-treated nitrocellulose filter was overlaid onto the agar surface and incubated for another 5 hours. The filter was marked, washed with TBS buffer (pH 8.0) at room temperature for 15 minutes, and blocked with 4% milk in PBS for 30 minutes. Pre-absorbed MGIS antiserum was added to the filter and allowed to react at room temperature overnight. The filter was subsequently washed in TBS at room temperature for 10 minutes, three times. Goat anti-cat IgA polyclonal antibody labeled with HRP (available from Bethyl Laboratories, Inc.) was diluted 1:300 in TBS buffer and incubated with the filter at room temperature for 2 hours. The filter was washed and developed using 4-chloro-1-naphthol substrate and H₂O₂. Thirteen of the 23 clones were identified as positive for expressing antigen recognized by IgA in the MGIS antiserum.

DNA Sequencing

the TA vector using the TA cloning kit (available from Invitrogen). Individual clones were PCR amplified using the T3 and T7 vector-specific primers. The DNA fragments produced by PCR amplification were gel electrophoresed on a 1% agarose gel, and gel purified using a Qiagen Gel Purification kit (available from Qiagen). Plasmid DNA was purified using the 5 prime 3 prime Perfect Plasmid DNA Preparation kit (available from 5 Prime 3 Prime Inc., Boulder, CO). DNA sequencing was carried out on six of the T. gondii-specific DNA inserts using a Prizm dideoxy termination kit (available from Perkin Elmer) on an ABI 377 DNA sequencer (available from Applied Biosystems). TA sense and TA antisense oligonucleotide primers were used for DNA sequencing, and insert-specific oligonucleotide primers were used to generate internal fragment sequences. The only variation from this general protocol was in the case of MGIS4-4,

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where the Erase a Base system (available from Promega) was used to generate plasmids containing deleted fragments in order to facilitate sequencing. The primers used for sequencing each of the inserts were the following:

The primers used in sequencing MGIS4-2 are herein referred to as SEQ ID NO:275, SEQ ID NO:276, SEQ ID NO:277, SEQ ID NO:278, SEQ ID NO:279, SEQ ID NO:280, and SEQ ID NO:281. The primers used in sequencing MGIS4-4 are herein referred to as SEQ ID NO:285, SEQ ID NO:286, SEQ ID NO:287, SEQ ID NO:288, SEQ ID NO:289, SEQ ID NO:290, and SEQ ID NO:291. The primers used in sequencing MGIS4-8 are herein referred to as SEQ ID NO:295, SEQ ID NO:296, SEQ ID NO:297, SEQ ID NO:298, SEQ ID NO:209, SEQ ID NO:300, SEQ ID NO:301, SEQ 10 ID NO:302, SEQ ID NO:303, SEQ ID NO:304, and SEQ ID NO:305. The primers used in sequencing MGIS6-5 are herein referred to as SEQ ID NO:309 and SEQ ID NO:310. The primers used in sequencing MGIS6-2 are herein referred to as SEQ ID NO:314, SEQ ID NO:315, SEQ ID NO:316, SEQ ID NO:317, SEQ ID NO:318, SEQ ID NO:319, SEQ ID NO:320, SEQ ID NO:321, SEQ ID NO:322, SEQ ID NO:323, SEQ ID NO:324, 15 and SEQ ID NO:325. And the primers used in sequencing MGIS1-3 are herein referred to as SEQ ID NO:314, SEQ ID NO:315, SEQ ID NO:316, SEQ ID NO:317, SEQ ID NO:318, SEQ ID NO:319, SEQ ID NO:320, SEQ ID NO:321, SEQ ID NO:322, SEQ ID NO:323, SEQ ID NO:324, and SEQ ID NO:325 (note that the same primers were used 20 for sequencing MGIS6-2 and MGIS1-3).

PCR Amplification of Feline and T. gondii DNA With Clone-specific Primers

The IgA selected MGIS clones were shown to be Toxoplasma specific by PCR amplification analysis. The following different cDNA samples were tested for the presence of DNA representing (ach of the six different IgA-selected nucleic acid molecules: a) uninfected cat gut (UCG); b) infected cat gut (ICG); c) T. gondii tachyzoite (TgTz); d) Toxoplasma bradyzoite (TgBz); and e) Toxoplasma genomic DNA (TgTz DNA). The preparation of UCG, ICG, Toxoplasma tachyzoite and bradyzoite cDNA was as described above. Toxoplasma genomic DNA was isolated from tachyzoites by phenol/chloroform/isoamylalcohol pH 8.0 extraction.

Oligonucleotide sense and anti-sense primers specific to each of five MGIS-selected nucleic acid molecules were synthesized and used as primers in the PCR amplification reactions. The reaction condition were: 95 °C for 10 min., followed by 35 cycles of 95 °C for 30 sec., 58 °C for 30 sec., 72 °C for 40 sec; this was followed by 75 °C for 5 min. afterwards to complete the reaction. The amount of the different templates used in the PCR reactions (~3-30 ng of DNA), was empirically determined by comparison with a PCR amplified *Toxoplasma* tubulin gene product standard generated with each template. The oligonucleotide primers and the size of the expected products are listed in Table 13, below.

10 Table 13.

MGIS Clone	Sense Primer Position: Sequence	Anti-Sense Primer Position: Sequence	Product Size (bp)		
1-3	1513: SEQ ID NO: 319	1858: SEQ ID NO: 320	346		
4-2	168: SEQ ID NO: 276	594: SEQ ID NO: 279	427		
4-4	455: SEQ ID NO: 285	775: SEQ ID NO: 290	331		
4-8	2018: SEQ ID NO: 300	2310: SEQ ID NO: 301	293		
6-2	1301: SEQ ID NO: 319	1646: SEQ ID NO: 320	346		

The oligonucleotide primers specific for each of the five MGIS-selected nucleic acid molecules PCR amplified products only when the template DNA contained *Toxoplasma* DNA. There were no PCR amplified products in this assay when the template DNA was UCG cDNA. These results confirm the *T. gondii* origin of the MGIS-selected nucleic acid molecules.

Sequence Analysis

Homology searches of a non-redundant protein database were performed on all
six MGIS-selected nucleic acid molecules, translated into all six reading frames, using
the BLASTX program available through the BLASTTM network of the National Center
for Biotechnology Information (NCBI) (National Library of Medicine, National Institute
of Health, Baltimore, MD). This database includes SwissProt + PIR + SPupdate +
GenPept + GPUpdate + PDB databases. In addition, BLASTN homology searches were

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performed on these sequences using the NCBI databases including the non-redundant database of GenBank EST, and genembl. In all cases, the default parameters for the homology programs were used.

The highest scoring match of the homology search (BLASTX) of translation products of the nucleic acid sequence SEQ ID NO:282 (MGIS4-2) was to GenBank[™] Accession No. prf 2208369A, a *Homo sapiens* signal peptidase 12kD subunit protein. The protein encoded by nucleic acid residues 742-945 of MGIS4-2 (SEQ ID NO:282) showed about 44% identity to amino acid residues 12 to 79 of the protein represented by GenBank[™] Accession No. prf 2208369A. At the nucleotide level, SEQ ID NO:282 showed 97% identity over 353 nt with te sequence represented by GenBank[™] Accession No. W0680 (TgESTzy81e12.r1), an EST fragment isolated from *T. gondii* tachyzoite cDNA. The homology spans the region from nt 748 to nt 1097 of SEQ ID NO:282, and nt 15 to 365 of GenBank[™] Accession No. W0680. There were no other significant homology matches to SEQ ID NO:282 nucleic acid sequence.

The highest scoring matches of the homology search (BLASTX) of translation products of the nucleic acid sequence SEQ ID NO:292 (MGIS4-4) were to proteins described as elongation factor 1-gamma, with the highest match to the sequence represented by GenBank™ Accession No. gi 2160158, described as "a protein similar to elongation factor" The protein encoded by residues 47-1222 of SEQ ID NO:292 showed about 37% identity to amino acid residues 5-414 of the protein represented by GenBank™ Accession No. gi 2160158. At the nucleotide level SEQ ID NO:292 showed 94% identity over 413 nt with an EST fragment, GenBank™ Accession No. N81326 (TgESTzy40a12.r1), an EST fragment isolated from *T. gondii* cDNA. The homology spans the region from nt 420 to nt 832 of SEQ ID NO:292, and nt 15 to 427 of GenBank™ Accession No.N81326. In addition, SEQ ID NO:292 showed 99% identity over 187 nt with an EST fragment, GenBank™ Accession No. W05869 (TgESTzy85a09.r1), an EST fragment isolated from *T. gondii* cDNA clone. The homology spans the region from nt 757 to nt 943 of SEQ ID NO:292, and nt 62 to 248 of GenBank™ Accession No.W05869.

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The highest scoring match of the homology search (BLASTX in the genembl database) of translation products of the nucleic acid sequence SEQ ID NO:329 (MGIS1-3) was to Herpesvirus Saimiri complete genome, represented by GenBank[™] Accession No. X64346. The amino acid residues 777 to 1432 of the protein encoded by reading frame +2 of SEQ ID NO:329 showed about 36% identity to amino acid residues 106974 to 106517 of the protein represented by GenBank[™] Accession No. X64346. At the nucleotide level, SEQ ID NO:329 showed 94% identity over 578 nt with an EST fragment, GenBank[™] Accession No. AA520348 (TgESTzz69d04.r1), an EST fragment isolated from *T. gondii* bradyzoite cDNA. The homology spans the region from nt 1334 to 1910 of SEQ ID NO:329, and nt 5 to 571 of GenBank[™] Accession No. AA520348.

The highest scoring match of the homology search (BLASTN of the non-redundant databases, GenBank+EMBL+DDBJ+PDB) of SEQ ID NO:311 (MGIS6-5) was to a *T. gondii* lactate dehydrogenase gene, represented by GenBank[™] Accession No. TGU35118. SEQ ID NO:311 showed 99% identity over 1619 nt.

The highest scoring match of the homology search (BLASTX in the genembl database) of translation products of the nucleic acid sequence SEQ ID NO:326 (MGIS6-2) was to Herpesvirus Saimiri complete genome, represented by GenBank[™] Accession No. X64346. Amino acid residues 751 to1206 encoded by SEQ ID NO:326 showed about 36% identity to amino acid residues 106972 to 106517 of the protein represented by GenBank[™] Accession No. X64346. At the nucleotide level, SEQ ID NO:326 showed 96% identity over 247 nucleotides with an EST fragment, GenBank[™] Accession No. AA520348 (TgESTzz69d04.r1), an EST fragment isolated from *T. gondii* bradyzoite cDNA. The homology spans the region from nt 890 to1136 of SEQ ID NO:326, and nt 144 to 390 of CenBank[™] Accession No. AA520348.

The highest scoring match of the homology search (BLASTX of the non-redundant GenBank CDS database including

Translations+PDB+SwissProt+SPupdate+PIR) of translation products of the nucleic acid sequence SEQ ID NO:306 (MGIS4-8) was to a rice 26S protease regulatory subunit 4 homolog (TAT-binding protein homolog 2), represented by GenBankTM Accession No.

P46466. 26S protease regulatory subunit 4 homologs representing other species also

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have high homology to a translation product of SEQ ID NO:306. The protein encoded by nucleic acid residues 465 to 1565 of SEQ ID NO:306 showed about 72% identity to amino acid residues 35 to 448 of the protein represented by GenBank™ Accession No. X64346. It should be noted a gap of 42 amino acids was required in the amino acid sequence encoded by SEQ ID NO:306 in order to achieve the sequence fit resulting in this high homology. At the nucleotide level, SEQ ID NO:306 showed 98% identity over 269 nucleotides with an EST fragment, GenBank™ Accession No. W35531 (TgESTzy90g01.r1), an EST fragment isolated from *T. gondii* cDNA. The homology spans the region from nt 668 to nt 936 of SEQ ID NO:326, and nt 23 to nt 291 of GenBank™ Accession No. W35531.

Example 16:

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This Example discloses the isolation and sequence analysis of a 1397 bp T. gondii nucleic acid molecule composed of four fragments isolated by subtractive selection from an infected cat gut cDNA library. Also described is an additional nucleic acid molecule representing the genomic DNA sequence immediately upstream (5') of, and overlapping, the genomic DNA sequence encoding the cDNA sequence.

A 1397 bp *T. gondii* nucleic acid molecule, denoted nTG₁₃₉₇ (the coding strand of which is herein referred to as SEQ ID NO:343, and the reverse complement of which is herein referred to as SEQ ID NO:345), is a composite of four overlapping PCR amplified products isolated from an infected cat gut (ICG) cDNA library. Specifically, a first 424 bp fragment (representing nucleotide positions 709-1132 of SEQ ID NO:343), was isolated after two rounds of selection using the PCR-Select™ Subtraction kit (available from Clontech, Palo Alto, CA), using day eight, *Rsa*I restriction enzyme digested ICG cDNA as tester, and similarly digested uninfected cat gut cDNA as driver DNA. Fragments enriched by the PCR-Select™ Subtraction selection process were digested with the restriction enzyme *Sma*I and cloned into *Sma*I site in the commercially available positive selection vector, QuanTox™ (available from Quantum Biotechnologies Inc., Laval, Quebec, Canada). The cloned inserts were subsequently sequenced using the oligonucleotide primers, T7 (TAATACGACTCACTATAGGG, herein referred to as SEQ ID NO:348) and T3 (ATTAACCCTCACTATAAGGGA, herein

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referred to as SEQ ID NO:347). A 424 bp T. gondii nucleic acid molecule, referred to herein as nTG_{424} , was isolated, cloned and sequenced by this method.

The orientation of nTG₄₂₄, as well as additional nucleic acid sequence representing cDNA sequence occurring downstream (3') of nTG₄₂₄ was determined as 5 follows. A 689 bp fragment including the 3'-end of the gene comprising nTG₄₂₄ was generated by PCR amplification of an ICG cDNA library constructed in the Uni-Zap XR insertion vector (available from Stratagene). The two primers used for this amplification reaction are represented by SEQ ID NO:358 (109ACAACGACCACGACATCAACTAC⁷³¹, derived from the sequence of nTG₄₇₄, also referred to as pRay8), and an adaptor oligonucleotide primer that hybridized to the 10 cDNA poly A tail (GGCCACGCGTCGACTACT₁₇ from BRL/GIBCO, Gaithersburg, MD, herein referred to as SEQ ID NO:364). The superscript numbers at the beginning and end of the primer sequences described herein represent the location of the primer sequence relative to nTG₁₃₉₇ (SEQ ID NO:343). A resulting 689bp T. gondii nucleic acid molecule (also referred to as nTG₆₈₉) was cloned into PCR2.1 (available from Invitrogen, 15 Carlsbad, CA), and sequenced using the M13 reverse oligonucleotide primers (CAGGAAACAGCTATGACC, herein referred to as SEQ ID NO:346) and the T7 oligonucleotide primer (SEQ ID NO:348). The sequence of nTG₆₈₉ revealed 266 bp of additional cDNA sequence (from 1133-1397 bp, relative to SEQ ID NO:343), with an overlap with nTG₄₂₄ from 709-1132 bp (relative to SEQ ID NO:343). There were three 20 nucleotide differences between the sequence data for nTG₄₂₄ and the sequence data for nTG₆₈₉. Instead of a "T", "C" and "T" nucleotide at positions 1159, 1166, and 1169 respectively, the sequence data for nTG₆₈₉ revealed a "C", "T", and "A" at those positions.

The remainder of the nucleic acid sequence of nTG₁₃₉₇ was determined in two PCR amplification steps using the ICG cDNA library as the template. The primers for the first PCR amplification were: a) an anti-sense oligonucleotide primer specific for nTG₄₂₄, having the sequence ⁹²⁹GTTGTCGTAGATGTCGTTGTAGTT⁹⁰⁶, and herein referred to as SEQ ID NO:359; and b) a Uni-Zap XR insertion vector-specific oligonucleotide primer (available from Stratagene, and referred to as Tp277) having the

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sequence, GGGAACAAAAGCTGGAGCTCCACC, and herein referred to as SEQ ID NO:354. In the first PCR amplification step, SEQ ID NO:359 and SEQ ID NO:354 were used to generate an 884 bp nucleic acid molecule, (825 bp of which was nTG₁₃₉₇-specific DNA sequence), that was then cloned into PCR2.1. The *T. gondii*-specific nucleic acid molecule is herein referred to as nTG₈₂₅. nTG₈₂₅ was sequenced using a TA sense oligonucleotide primer (having the sequence, CGAGCTCGGATCCACTAG, herein referred to as SEQ ID NO:350), and a TA anti-sense oligonucleotide primer (having the sequence, GCCAGTGTGATGGATATCTGCAG, herein referred to as SEQ ID NO:349), as well as a nTG₁₃₉₇-specific internal oligonucleotide primer having the sequence, ⁵⁶⁴GAGGAGATCGAACTTTGCTTGTGC⁵⁴¹, herein referred to as SEQ ID NO:361. Sequencing revealed that nTG₈₂₅ added an additional 604 bp to the sequence of nTG₁₃₉₇, from nucleotides 105-708 (relative to SEQ ID NO:343). nTG₈₂₅ overlapped with nTG₄₂₄ and nTG₆₈₉ from base 709-939 (relative to SEQ ID NO:343).

The primers for the second PCR amplification step were: a) an oligonucleotide

primer specific for nTG₄₂₄, having the sequence

225 AGAAGCGCCTTTGCGTTTCTACGT²⁰², herein referred to as SEQ ID NO:360; and

b) Tp277. These two primers were used to generate a 225 bp *T. gondii* DNA fragment, referred to as nTG₂₂₅. nTG₂₂₅ cloned into PCR2.1, and nucleotide sequenced with the TA oligonucleotide primers as above, thereby generating the sequence from nucleotides 1
104 of SEQ ID NO:343. Sequence analysis revealed that nTG₂₂₅ overlapped with previously isolated nTG₈₂₅ DNA sequence from base105-225, relative to SEQ ID NO:343.

The contiguous cDNA sequence of the overlapping fragments representing nTG₁₃₉₇ was determined (and referred to herein as SEQ ID NO:343), and sequence analysis of the composite molecule revealed an 867 bp coding region (referred to as nTG₈₆₇), assuming an initiation codon at position 238-240, and a stop codon at position 1102-1104 (relative to SEQ ID NO: 343). The coding strand of nTG₈₆₇ is herein referred to as SEQ ID NO:340, and the reverse complement is herein referred to as SEQ ID NO:342. Translation of the coding region of nTG₈₆₇ yields a 288 amino acid protein

herein referred to as PTg₂₈₈, the amino acid sequence of which is herein referred to as SEQ ID NO:341.

To confirm the DNA sequence in the predicted coding region of nTG₁₃₉₇, a PCR amplified fragment containing nucleotides 238 to 1271 was generated using an 5 oligonucleotide primer having the sequence, AAGGATAGGCGCCGCAGGTACC ²³⁸ATGGCAGGAAGGCAGGCGGCGTT²⁶⁰, herein referred to as SEQ ID NO:362, and an oligonucleotide primer having the sequence, ACCGCTCGAGAAGCTT ¹²⁷¹GAAGCCAAGACATCCCTTCGTGCA¹²⁴⁸, herein referred to as SEQ ID NO:363. The nucleotides in italics represent non-nTG₁₃₉₇ nucleotide sequence, and were present to attach convenient restriction sites to the PCR product. The resulting PCR fragment was 10 cloned into a eukaryotic expression vector, referred to as pDVacIII, and sequenced using two vector-specific oligonucleotide primers: a) Tp244, having the sequence. GGATGCAATGAAGAGGGCTC, and herein referred to as SEQ ID NO:352; and b) Tp245, having the sequence, AACTAGAAGGCACAGTCGAGGCTG, and herein 15 referred to as SEQ ID NO:353. The PCR fragment thus generated contained two nucleotide differences as compared with the previously determined cDNA sequence of nTG₁₃₉₇. Instead of an "A" at position 643, a "G" residue was found, and in place of a "T" at position 1187, a "C" residue was found. The resulting nucleotide change at position 643 altered the predicted encoded amino acid from an arginine to a glycine 20 residue. The change at position 1187 did not change the predicted amino acid sequence of nTG₁₃₉₇.

Genomic DNA sequence upstream of the gene comprising nTG₁₁₉. was determined by generating a 747 bp fragment by PCR amplification of the λ-EMBL-3 Sau3A partial Toxoplasma genomic library herein described. The primers used were SEQ ID NO:360 (representing nucleotides 202-225 in nTG₁₃₉₇) and a λ-EMBL-3-specific primer having the sequence, GGTTCTCTCCAGAGGTTCATTAC, and herein referred to as SEQ ID NO:351. The resulting DNA fragment was cloned in PCR2.1 and sequenced with TA oligonucleotide primers (SEQ ID NO:349, and SEQ ID NO:350) and two gene specific oligonucleotide primers, Tp310

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Tp311 (²⁴³CACGAAGCTGCATGTTCCAGCTAG²⁶⁵, herein referred to as SEQ ID NO:356). The sequence of the PCR fragment revealed a 647 bp DNA fragment, nTG₆₄₇, (herein referred to as SEQ ID NO:338, the reverse complement is herein referred to as SEQ ID NO:339, including 421 nucleotides of new genomic DNA sequence upstream of the 5' end of the cDNA sequence of Tg₁₃₉₇. The fragment contained 327 bp of genomic DNA sequence that overlapped with the cDNA sequence, SEQ ID NO:343 (in other words, bases 422-647 of the genomic DNA sequence, SEQ ID NO:338, overlap with bases 1-225 of the cDNA sequence, SEQ ID NO:343). There was a single nucleotide difference between the genomic and the cDNA sequences at position 118 of the cDNA sequence (SEQ ID NO:343), where there is a "G" in the genomic DNA sequence and an "A" at the equivalent position in the cDNA sequence.

SEQ ID NO:343 was shown to be T. gondii specific by PCR amplification analysis of various DNAs, using nTG₁₃₉₇-specific DNA primers to drive the reaction. The following cDNA samples were tested for the presence of nTG_{1397} DNA: a) uninfected cat gut (UCG), b) infected cat gut (ICG), c) T. gondii tachyzoite (TgTz), and d) Toxoplasma bradyzoite (TgBz). To generate UCG and ICG RNA, gut tissue samples from an uninfected cat and a cat 7 days post infection with T. gondii tissue cysts (1000 cysts) were processed by scraping and collecting the epithelial layer of gut cells on dry ice. Cells from UCG, ICG, and T. gondii tachyzoites and bradyzoites were solubilized by homogenization in TRI-reagent (available from Molecular Research Center Inc., Cincinnati, OH), and the homogenate passed through a 18/20/and 22 gauge needle 10 times each sequentially. After standing at room temperature for 5 min., 100 ul of bromochloropropane (available from Molecular Research Center Inc.)/ ml of TRI reagent was added, and the homogenate vortexed for 15 seconds. The sample was centrifuged at 14,000 rpm for 15 min. at 4°C, the aqueous layer collected, and RNA precipitated with one half volume of isopropanol. Contaminating genomic DNA was removed by digestion with 10 units of RNase free DNaseI (available from Boehringer Mannheim Corp.) at 37 °C for 30 min. The sample was then extracted with phenol/chloroform/isoamylalcohol, pH 6.0. The RNA was precipitated from the aqueous layer with ethanol and resuspended in diethylpyrocarbonate (available from

Sigma) treated water. cDNA was generated from total RNA using a commercially available RT-PCR kit (available from Stratagene).

Two nTG₁₃₉₇-specific oligonucleotide primers were used in the reaction: SEO ID NO:358, having the sequence, ⁷⁰⁹ACAACGACCACGACATCAACTAC⁷³¹, and SEO ID NO:357, having the sequence, 1114ACACTTTGGTCTAATCGAGGGTAG¹⁰⁹¹. The 5 reaction conditions were: 95 °C 12 min., followed by 3 cycles of 94 °C 30 sec., 70 °C 30 sec., 72 °C 60 sec., 3 cycles of 94 °C 30 sec., 67 °C 30 sec., 72 °C 60 sec., 3 cycles of 94 °C 30 sec., 65 °C 30 sec., 72 °C 60 sec., 6 cycles of 94 °C 30 sec., 63 °C 30 sec., 72 °C 60 sec., 25 cycles of 94 °C 30 sec., 59 °C 30 sec., 72 °C 60 sec., and a seven minute 10 extension at 75 °C to complete the reaction. The amount of template used in each PCR reaction (~3-30 ng of DNA), was empirically determined by comparison with a PCR amplified Toxoplasma tubulin gene product standard generated with each template. The PCR amplification reaction generated a 406 bp product only in the reactions containing tachyzoite and ICG cDNA template DNA, thereby confirming the T. gondii-specificity 15 of SEQ ID NO:343.

Sequence Analysis

Homology searches of a non-redundant protein database were performed on SEQ ID NO:340 (representing the coding region of nTG₁₃₉₇, translated in frame 1, using the BLASTP program available through the BLASTTM network of the National Center for Biotechnology Information (NCBI) (National Library of Medicine, National Institute of Health, Baltimore, MD). This database searched was PIR. In addition, a BLASTP homology search was performed on SEQ ID NO:341 (representing the amino acid sequence encoded by SEQ ID NO:340) using the NCBI database SwissProt. In all cases, the default parameters for the homology programs were used. Another homology search was run on SEQ ID NO:343 using the BLASTN search program and the database genembl.

When run against the PIR database, the highest scoring match of the homology search of translation products of the nucleic acid sequence SEQ ID NO:340 (the coding strand of the coding sequence) was to GenBankTM accession number A60095, a *Drosophila* larval glue protein precursor. Other significant homologies included

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homology to an African clawed frog mucin, and a promastigote surface antigen-2. When analyzed by the GCG program, using BESTFIT and default parameters, amino acid residues 145 to 281 of the protein encoded by SEQ ID NO:340 showed about 70% identity to amino acid residues 42 to 178 of the protein represented by GenBank™ accession number A60095. In addition, amino acid residues 153 to 282 of the protein encoded by SEQ ID NO:340 showed about 73% identity to amino acid residues 394 to 523 of the protein represented by GenBank™ accession number A45155 (African clawed frog mucin). When compared with the SwissProt database, the highest scoring match of the homology search of the amino acid sequence SEQ ID NO:341 (the protein encoded by SEQ ID NO:340) was to GenBankTM accession number Q05049, the African clawed frog mucin. These two amino acid sequences showed a 73% identity from amino acid 153 to 282 of SEQ ID NO:341 and amino acid 394 10 523 of the amino acid sequence represented by GenBank™ accession number Q05049. A comparison of SEQ ID NO:343 (the cDNA coding strand) using the BLASTN search program and the database genembl revealed a 76% nucleic acid sequence identity to a D. discoideum protein kinase, GenBank™ accession number M38703. This identity was between nt 765 to 1058 of SEQ ID NO:343 and nt 772 to 1065 of the sequence represented by GenBank[™] accession number M38703. In addition, a BLASTN comparison SEQ ID NO:343 with the non-redundant GenBank™ database including GenBank EMBL+DDBJ+PDB revealed an 89% identity between nucleic acid residues 779 to 902 of SEQ ID NO:343 and nt 2150 to nt 2273 of the nucleic acid sequence represented by GenBank™ accession number DDDU86962.

Example 17:

This example describes the induction of humoral and cellular responses in cats by proteins expressed by the *T. gondii* nucleic acid molecules of the present invention. Protein immunization with *T. gondii* recombinant protein and several different adjuvants induced both antibodies and T cell proliferative responses in cats. DNA immunization of cats with plasmid constructs expressing *T. gondii* immunogenic proteins of the present invention also induced antibody responses.

Protein Immunization

Protein immunization of cats was carried out with three primary subcutaneous immunizations at intervals of four weeks (prime at week 0 and boosts at weeks 4 and 8) using 50 µg protein per injection in adjuvant. The primary antigen was OC-22, which was purified as a HIS fusion protein from E. coli. The experimental groups were as follows: two cats were immunized with OC-22 protein in alum, two cats were immunized with OC-22 protein in polyphosphazine (PCPP), and two cats were immunized with OC-22 protein in BAYER1005 (Stunkel, K.G., et al., in Cellular Basis of Immune Modulation, 1989, pp. 575-579, incorporated herein by reference in its entirety). One cat was injected with two different antigens in BAYER1005: 50 ug of OC-22 and 12 ug of protein 4499-9. One control cat was injected with saline.

Whole blood was collected from all of the animals at intervals before and after the immunizations. Mononuclear cells were selected from the blood for T cell proliferation analysis (see blow) and the remaining plasma processed for detection of humoral responses. The presence of antibody was determined by western blot analysis and by ELISA using recombinant purified antigens. The western blot analysis was more sensitive at detecting a positive or negative response, while the ELISA provided a more quantitative comparison of the cat's responses to the immunogenic proteins.

Western blot analysis was performed on Recombinant purified OC-22 protein was loaded at 2 ug per lane and blotted to nitrocellulose. Samples were from preimmune cats and cats at 1, 3, and 5 weeks after immunization. Recombinant purified OC-22 protein was loaded at 2 ug per lane and blotted to nitrocellulose. Analysis of the sera collected at three weeks following the first immunization demonstrated that all seven cats responded positively to OC-22 protein. Both anti-cat IgG and anti-cat IgA 25 were used as secondary antibodies (on separate blots). The westerns showed that OC-22 protein elicited both IgA and IgG responses, although the IgA response was not as strong as the IgG response. The ELISA titers were monitored throughout the immunization regimen. The sera collected at week eight and a half, immediately following the second boost had detectable ELISA titers equal to or greater than 1:10,000 for all seven cats. These analyses did not demonstrate any apparent differences between the cats immunized with different adjuvants. The single cat immunized with 12 ug of 4499-9

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protein was not positive to 4499-9 protein by either western blot analysis or ELISA, although the same cat demonstrated immune responses to OC-22 that were comparable to the other cats in the study.

Cellular responses to the recombinant *T. gondii* OC-22 protein were tested by *in vitro* proliferation of isolated peripheral blood mononuclear cells (PBMC) to purified protein at concentrations ranging from 0.5 to 8 μg/ml. At higher concentrations of protein, non-specific stimulation was evident, making interpretation difficult, but at lower concentrations of antigen, distinct differences were seen between cats. One week after the first boost, T cells from all of the cats in either the PCPP or BAY R1005 adjuvant groups demonstrated stimulation indices (SI) greater than 3. Cells from the PBS control and two alum group cats did not show any proliferative responses. Peak proliferative responses were seen one week after each boost, with the highest responses observed after the first boost. The cats immunized with protein in PCPP had the highest responses, followed by the cats immunized with protein in BAY R1005. The responses observed at 0.5 μg antigen per ml were lower than the responses observed at higher doses, but correlated well with the results observed at 2 ug/ml (data not shown). All of the immunized cats responded to antigen, at some point during the experiment, with an SI level above 3.

DNA Immunization

Cats were immunized with the recombinant eukaryotic expression vector, pDVac II, encoding *T. gondii* nucleic acid molecules encoding the immunogenic proteins OC-2, OC-22, and Tg-50. The pDVacII vector contains the CMV promoter and intron A sequences. The protein expressed by this vector includes the *T. gondii* antigen of interest, fused at the 5 prime end to the tissue plasminogen activator signal sequence and fused at the three prime end with both a stretch of poly histidines and an amino acid epitope from the mammalian *myc* gene. Fifteen cats were divided into four experimental groups: three cats received saline (cats 1, 8, and 16), four cats received DNA encoding OC-2 (cats 2, 5, 9, and 15), four cats received DNA encoding OC-22 (cats 3, 6, 10, and 12), and four cats received a combination of DNA encoding OC-22, OC-22, and Tg-50 (cats 4, 7, 13, and 14). Each cat was injected intramuscularly with a total 300 ug of DNA at two sites per immunization. The combined formulation included 300 ug of each

plasmid per injection. The cats were given one injection and then at eight weeks received a boost.

The serum samples collected at six weeks after the primary immunization were analyzed. Two out of eight cats immunized with OC-2 DNA were shown to sero convert to antibody positive to OC-2 protein by western blot analysis. None of the sera collected at this time from the cats immunized with OC-22 or Tg-50 DNA were positive by western blot analysis to OC-22 or Tg-50 protein respectively. When sera collected one week following the boost (week 9) were analyzed by western blots, seven of eight cats immunized with OC-2 were positive to OC-2, six of eight cats immunized with OC-22 were positive to OC-22, and one of four cats immunized with Tg-50 were positive to Tg-50. Similar to the western blot analysis for the protein immunogenicity study described above, faint IgA responses from all of the OC-22 sero-positive animals could be observed. ELISA analysis of sera taken one week after the boost indicated that four out of eight cats immunized with OC-2 and four of eight cats immunized with OC-22 had midpoint titers greater then 1:1000.

The T cell analysis demonstrated positive proliferative responses to several antigens, however the data were difficult to interpret. Cells isolated from two cats immunized with the OC-22 gene and one cat immunized with the OC-2 gene each demonstrated significant SI responses. However, the same cells from each of these cats were also stimulated by the other recombinant antigen; i.e. cells from OC-22-injected cats responded to OC-2 protein and cells from OC-2-injected cats responded to OC-22 protein. Sera from these animals did not react with the poly histidine or *myc* fusions on other control fusion proteins. This inability to demonstrate strong proliferative responses in PBMC is consistent with other results observed while exploring the induction of proliferative responses in T cells from DNA immunized cats. Cat peripheral blood is a poor source of responsive T cells.

Analysis of Oocyst Shedding in Protein an DNA immunized cats:

Analysis of oocysts shed following tissue cyst challenge of cats in both the protein and DNA immunogenicity studies showed no significant difference in oocyst shedding between any of the test groups and the control within each study. However, the number of animals in these studies varied between two and four per group, and thus

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this result is statistically meaningless. However, significant reduction, i.e., greater than several logs of total oocysts, was not observed in this experiment.

Example 18:

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This example describes immunization of cats with nucleic acid molecules encoding immunogenic *T. gondii* proteins, and subsequent challenge of the immunized cats.

Immunization Protocol:

The following set of conditions were used for the delivery of DNA-coated gold particles to cats: 1.25 ug of DNA was delivered per shot by Gene Gun (available from Biorad). 1.6 micron gold particles were used in the presence of 0.05 mg/ml PVP (polyvinyl pyrrolidine, 360 kD). The micro-carrier loading quantity was 0.5 mg DNA/cartridge, while the DNA loading ratio was 2.5 ug DNA/mg gold. The animals were anesthetized and shaved at the points of contact with the gun. A total of six shots were delivered to the animal for each immunization: three shots to the inner thigh at 300 psi and three shots to the lower side of the abdomen at 600 psi. The immunization regimen consisted of one prime and two boosts at six week intervals. Tissue cyst challenge was performed two weeks following the second boost. The challenge was with 1000 mouse brain-derived tissue cysts.

The plasmid containing the human growth hormone (hGH) gene was used in the control groups and as a marker in the other groups in all studies. In most control groups, the hGH plasmid was diluted to a concentration similar to that in the test groups. Humoral immune responses to the gene product were measured with an ELISA assay, and cellular responses were measured using hGH protein.

First immunogenicity study: The first immunization was followed by a challenge of 1000 mouse brain derived tissue cysts fourteen weeks later. Sample collection was terminated three weeks after that. There were four groups of five animals per group, as follows: Group 1: Control, hGH (0.125 ug/shot), pDVacIII (1.125 ug/shot) This group received one prime and two boosts, at 0, 6 and 12 weeks, respectively. Group 2: OC-22 in pDVacIII (1.25 ug/shot). This group received one prime and two boosts, at 0, 6 and 12 weeks, respectively. Group 3: hGH (0.125 ug/shot), 9 Toxoplasma nucleic acid molecules OC-2, OC-22, OC-13, OC-14, Tg-41, Tg-45, Tg-50, 4604-3, and 4CQA11

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(0.125 ug/shot). This group received one prime and two boosts, at 0, 6 and 12 weeks, respectively. Group 4: hGH (0.125 ug/shot), the same DNA as in Group 3 (9) Toxoplasma nucleic acid molecules), but this group received one prime and one boost, at 6 and 12 weeks, respectively. ELISA analysis for hGH sero conversion using sera collected throughout the study demonstrated that five of five cats in Group 1 were positive (i.e., demonstrated an end point titer > 1,000). Three of five animals in Group 3 were sero-positive to hGH. ELISA analysis for sero conversion to OC-22 protein using sera from Group 2 and Group 3 indicated that three of five and zero of five cats were positive respectively. These data suggest that competition from the other plasmids reduced the rate of sero conversion to an individual plasmid. In all cases positive titers did not occur until after the first boost. Specific-T cell proliferative responses using PBMC from animals in each group were not observed. Using the B1 gene-based PCR ELISA herein described, the average number of oocysts shed for each group was: Group 1, 1.03e8; Group 2, 1.11e8; Group 3, 5.79e7 and Group 4, 8.83e7. Statistical analysis of the data indicated no significant difference between the test groups and the control.

Second immunogenicity study:

The first immunization for this study was followed by a challenge of 1000 mouse brain derived tissue cysts fourteen weeks later. Sample collection was terminated three weeks after that. There were four groups of five animals per group, and all animals received one prime and two boosts. Group 2 consisted of DNA representing 18 nucleic acid molecules of the present invention. Group 3 represent 14 additional nucleic acid molecules of the present invention. Group 4 was a combination of both of these groups. The specific nucleic acid molecules and concentrations used in the immunizations were as follows: Group 1: Control, hGH (0.083 ug/shot), pDVacIII (1.125 ug/shot). Group 2: hGH (0.070 ug/shot), 18 Toxoplasma nucleic acid molecules (BZ1-2, 4604-2, 4604-62, 4CQA27, 4CQA29, 4CQA21, 4CQA27, 4604-62, Q2-4, R8050-6, Tg50, M2A1, M2A5, M2A7, M2A11, M2A19, M2A22, M2A29) (0.070 ug/shot). Group 3: hGH (0.083 ug/shot), 14 Toxoplasma nucleic acid molecules (M2A3, M2A21, M2A18, 30 M2A20, M2A24, M2A6, Q2-9, Q2-10, Q2-11, 4604-63, 4604-17, 4604-69, 4604-54,

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4CQA19) (0.083 ug/shot). Group 4: hGH (0.040 ug/shot), 32 Toxoplasma nucleic acid molecules (BZ1-2, 4604-2, 4604-62, 4CQA27, 4CQA29, 4CQA21, 4CQA27, 4604-62, Q2-4, R8050-6,Tg-50, M2A1, M2A5, M2A7, M2A11, M2A19, M2A22, M2A29,M2A3, M2A21, M2A18, M2A20, M2A24, M2A6, Q2-9, Q2-10, Q2-11, 4604-63, 4604-17, 4604-69, 4604-54, 4CQA19) (0.040 ug/shot).

The ELISA analysis of antibody to hGH protein demonstrated that two of five, three of five, zero of five, and two of five animals seroconverted in Groups 1, 2, 3, and 4 respectively. Using low amounts of hGH plasmid in the presence of eighteen or thirty-two additional plasmids containing nucleic acid molecules of the present invention still induced sero conversion in several animals per group. This observation suggests that there is not a strict reduction in the production of antibodies when a gene is injected with several other constructs.

What is claimed is:

- 1. An isolated nucleic acid molecule encoding an immunogenic *T. gondii* protein that can be identified by a method comprising: a) immunoscreening a library selected from the group consisting of a *T. gondii* genomic expression library and a *T. gondii* cDNA expression library with an antiserum, wherein said antiserum is selected from the group consisting of antiserum raised against *T. gondii* oocysts, antiserum raised against *T. gondii* bradyzoites, antiserum raised against *T. gondii* infected cat gut, and antiserum isolated from a cat immune to *T. gondii* infection; and b) identifying a nucleic acid molecule in said library that expresses a protein that selectively binds to an antibody in said antiserum.
- A method to isolate a nucleic acid molecule encoding an immunogenic T. gondii protein, said method comprising: a) immunoscreening a library selected from the group consisting of a T. gondii genomic expression library and a T. gondii cDNA expression library with an antiserum selected the group consisting of antiserum raised against T. gondii oocysts, antiserum raised against T. gondii bradyzoites, antiserum raised against T. gondii infected cat gut, and antiserum from a cat immune to T. gondii infection; b) identifying a nucleic acid molecule in said library that expresses a protein that selectively binds to an antibody in said antiserum; and c) recovering said nucleic acid molecule from said library.
- 3. An isolated immunogenic *T. gondii* protein that can be identified by a method comprising: a) immunoscreening a library selected from the group consisting of a *T. gondii* genomic expression library and a *T. gondii* cDNA expression library with an antiserum, wherein said antiserum is selected from the group consisting of antiserum raised against *T. gondii* oocysts, antiserum raised against *T. gondii* bradyzoites,
- antiserum raised against *T. gondii* infected cat gut, and antiserum isolated from a cat immune to *T. gondii* infection; and b) identifying a protein expressed from said library that selectively binds to antibodies in said antiserum.
 - 4. An isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID

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- NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID 10 NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:338, SEQ ID NO:340, and SEQ ID NO:343.
- An isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising a nucleic acid molecule selected from the group consisting of nTG2₃₃₉, nTG4₅₂₆, nTG4₁₄₇₈, nTG5₆₅₇, nTG5₁₀₂₉, nTG6₄₂₅, nTG7₄₁₇, nTG8₅₀₇, nTG9₇₁₈, nTG10₄₄₁, nTG11₄₂₈, nTG13₂₈₂, nTG15₃₀₄, nTG16₂₈₄, nTG17₆₉₀, nTG18₃₁₃, nTG19₃₈₉, nTG21₅₄₈, nTG22₃₁₀, nTG23₂₂₀, nTG24₆₄₂, nTG25₃₈₁, nTG26₄₃₂, nTG27₂₈₂, nTG28₄₆₆, nTG30₅₃₉, nTG31₁₂₃₃, nTG32₄₁₁, nTG33₄₄₁, nTG34₄₉₁, nTG35₃₈₇, nTG36₄₁₇, nTG37₄₁₆, nTG38₅₀₀, nTG40₃₂₁, nTG41₅₁₃, nTG42₅₂₈, nTG43₃₇₅, nTG44₅₄₃, nTG45₅₇₃, nTG46₁₈₃₅, nTG48₆₀₄, nTG48₅₄₉, nTG49₂₇₀, nTG50₃₀₆, nTG51₈₀₄, nTG52₈₆₇, nTG53₁₄₃₄, nTG64₆₈₀, nTG55₂₉₆, nTG56₇₂₃, nTG57₂₇₀, nTG58₅₀₃, nTG60₃₂₂, nTG61₃₉₀, nTG62₆₉₉, nTG63₄₁₉, nTG64₃₀₃, nTG65₆₉₆, nTG66₁₇₃, nTG67₃₆₉, nTG68₅₆₆, nTG69₆₁₆, nTG70₇₆₂, nTG71₂₃₆, nTG72₅₆₉, nTG73₂₃₂, nTG74₂₇₆, nTG75₃₀₉, nTG76₅₃₄, nTG76₄₂₃, nTG77₃₂₇,
 - 6. An isolated immunogenic protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising the

nTG78444 and nTG79928.

complement of a nucleic acid sequence selected from the group consisting of SEO ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEO ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEO ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEO ID 10 NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID 15 NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:340, and SEQ ID NO:343.

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7. An isolated immunogenic protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising a nucleic 20 acid molecule selected from the group consisting of nTG2₃₃₉, nTG4₁₄₇₈, nTG4₁₄₇₈. nTG5₆₅₇, nTG5₁₀₂₉, nTG6₄₂₅, nTG7₄₁₇, nTG8₅₀₇, nTG9₇₁₈, nTG10₄₄₁, nTG11_{4:4}, nTG13₂₈₂, nTG15₃₀₄, nTG16₂₈₄, nTG17₆₉₀, nTG18₃₁₃, nTG19₃₈₉, nTG21₅₄₈, nTG22₃₁₀, nTG23₂₂₀. $nTG24_{642}$, $nTG25_{381}$, $nTG26_{432}$, $nTG27_{282}$, $nTG28_{466}$, $nTG30_{539}$, $nTG31_{1231}$, $nTG32_{411}$ 25 nTG33₄₄₁, nTG34₄₉₁, nTG35₃₈₇, nTG36₄₁₇, nTG37₄₁₆, nTG38₅₀₀, nTG40₃₂₁, nTG41₅₁₃. nTG42₅₂₈, nTG43₃₇₅, nTG44₅₄₃, nTG45₅₇₃, nTG46₁₈₃₅, nTG48₆₀₄, nTG48₄₄, nTG49₂₇₀, $nTG50_{306}, nTG51_{804}, nTG52_{867}, nTG53_{1434}, nTG54_{680}, nTG55_{296}, nTG56_{733}, nTG57_{270},$ nTG58₅₀₃, nTG60₃₂₂, nTG61₃₉₀, nTG62₆₉₉, nTG63₄₁₉, nTG64₃₀₃, nTG65₆₉₉, nTG66₁₇₃, nTG67₃₆₉, nTG68₅₆₆, nTG69₆₁₆, nTG70₇₆₂, nTG71₂₃₆, nTG72₅₆₉, nTG73₂₃₂, nTG74₂₇₆, nTG75₃₀₉, nTG76₅₃₄, nTG76₄₂₃, nTG77₃₂₇, nTG78₄₄₄ and nTG79₉₂₈. 30

A composition to inhibit T. gondii oocyst shedding in a cat due to infection with 8. T. gondii, said composition comprising a compound selected from the group consisting of: an isolated immunogenic T. gondii protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising the complement of a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID 10 NO:54, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID 15 NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID 20 NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:340, and SEQ ID NO:343; an isolated antibody that selectively binds to said immunogenic T. gondii protein; and an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, 25 SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:57, SEQ ID 30

NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:340, and SEQ ID NO:343.

A method to inhibit T. gondii oocyst shedding in a cat due to infection with T. 9. gondii, said method comprising administering to said cat a composition comprising a compound selected from the group consisting of: a) an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising a nucleic 15 acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID 20 NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID 25 NO:91, SEO ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID 30

NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:282, SEQ ID NO:292, SEQ ID NO:306, SEQ ID NO:311, SEQ ID NO:338, SEQ ID NO:340, and SEQ ID NO:343. b) an isolated immunogenic T. gondii protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising the complement of a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30. SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID 10 NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID 15 NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID 20 NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:282. SEQ ID NO:292, SEQ ID NO:306, SEQ ID NO:311, SEQ ID NO:338, SEQ ID NO:340, and SEQ ID NO:343; and c) an isolated antibody that selectively binds to said immunogenic T. gondii protein. 25

- 10. The invention of Claim 1, 2 or 3 wherein said antiserum isolated from a cat immune to *T. gondii* infection is enriched for antibodies to *T. gondii* gametogenic stages.
- 11. An isolated antibody that selectively binds to a protein as set forth in Claim 3, 6 or 7.

- 12. The nucleic acid molecule of Claim 1, 4 or 5, wherein said nucleic acid molecule hybridizes under stringent hybridization conditions with a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEO ID NO:1. SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEO ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEO ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:50, SEQ ID NO:59, SEO ID 10 NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEO ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEO ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEO ID 15 NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:340, and 20 SEQ ID NO:343.
 - 13. The nucleic acid molecule of Claim 4 or 5, wherein said nucleic acid molecule encodes an immunogenic *T. gondii* protein.
- 14. The nucleic acid molecule of Claim 1, 4 or 5, wherein said nucleic acid molecule comprises a nucleic acid sequence that is at least about 75% identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID

- NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:340, and SEQ ID NO:343.
 - 15. The nucleic acid molecule of Claim 1, 4 or 5, wherein said nucleic acid molecule is selected from the group consisting of: a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ
- ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID
 - NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID
- NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID
- 30 NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:282, SEQ ID NO:292, SEQ ID

NO:306, SEQ ID NO:311, SEQ ID NO:338, SEQ ID NO:340, and SEQ ID NO:343.; and a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEO ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEO ID 5 NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEO ID NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEO ID 10 NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEO ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID 15 NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:282, SEQ ID NO:292, SEQ ID NO:306, SEQ ID 20 NO:311, SEQ ID NO:338, SEQ ID NO:340, and SEQ ID NO:343...

- 16. The nucleic acid molecule of Claim1, 4 or 5, wherein said nucleic acid molecule encodes a protein comprising an amino acid sequence that is at least about 75% identical to an amino acid sequence encoded by a nucleic acid molecule selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID
- NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID

NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:85. SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95. SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:282, SEQ ID NO:292, SEQ ID NO:306, SEQ ID NO:311, SEQ ID NO:338, SEQ ID NO:340, and SEQ ID NO:343.

- 17. The nucleic acid molecule of Claim 1, 4 or 5, wherein said nucleic acid molecule hybridizes under stringent hybridization conditions with a nucleic acid molecule comprising a nucleic acid sequence encoding a protein comprising an amino acid
- sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID
- 20 NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID
- NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:140, SEQ ID NO:142, SEQ ID NO:144, SEQ ID NO:266, SEQ ID NO:268, SEQ ID NO:270, SEQ ID NO:272, SEQ ID
- 30 NO:274, SEQ ID NO:283, SEQ ID NO:293, SEQ ID NO:307, SEQ ID NO:312, and SEQ ID NO:341.

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- The nucleic acid molecule of Claim 1, 4 or 5, wherein said nucleic acid molecule 18. is selected from the group consisting of: a nucleic acid molecule comprising a nucleic acid sequence that encodes a protein having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEO ID NO:8, SEO 5 ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEO ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:29, SEO ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEO ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEO ID 10 NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:71, SEO ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEO ID NO:83, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEO ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEO ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID 15 NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:140, SEQ ID NO:142, SEQ ID NO:144, SEQ ID NO:266, SEQ ID NO:268, SEQ ID NO:270, SEQ ID NO:272, SEQ ID NO:274, SEQ ID NO:283, SEQ ID NO:293, SEQ ID NO:307, SEQ ID NO:312, and SEQ ID NO:341; 20 and a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule encoding a protein having any of said amino acid sequences.
 - 19. The nucleic acid molecule of Claim1, 4 or 5, wherein said nucleic acid molecule comprises an oligonucleotide.
- A recombinant molecule comprising a nucleic acid molecule as set forth in Claim
 1, 4 or 5, operatively linked to a transcription control sequence.
 - 21. A recombinant virus comprising a nucleic acid molecule as set forth in Claim 1, 4 or 5.
 - 22. A recombinant cell comprising a nucleic acid molecule as set forth in Claim 1, 4 or 5.

- 23. The nucleic acid molecule of Claim 5, wherein said nucleic acid molecule hybridizes under stringent hybridization conditions with a nucleic acid molecule selected from the group consisting of nTG2₃₃₉, nTG4₅₂₆, nTG4₁₄₇₈, nTG5₆₅₇, nTG5₁₀₂₉, nTG6₄₂₅, nTG7₄₁₇, nTG8₅₀₇, nTG9₇₁₈, nTG10₄₄₁, nTG11₄₂₈, nTG13₂₈₂, nTG15₃₀₄, nTG16₂₈₄, nTG17₆₉₀, nTG18₃₁₃, nTG93₃₈₉, nTG21₅₄₈, nTG22₃₁₀, nTG23₂₂₀, nTG24₆₄₂, nTG25₃₈₁, nTG26₄₃₂, nTG27₂₈₂, nTG28₄₆₆, nTG30₅₃₉, nTG31₁₂₃₃, nTG32₄₁₁, nTG33₄₄₁, nTG34₄₉₁, nTG35₃₈₇, nTG36₄₁₇, nTG37₄₁₆, nTG38₅₀₀, nTG40₃₂₁, nTG41₅₁₃, nTG42₅₂₈, nTG43₃₇₅, nTG44₅₄₃, nTG45₅₇₃, nTG46₁₈₃₅, nTG48₆₀₄, nTG48₅₄₉, nTG49₂₇₀, nTG50₃₀₆, nTG51₈₀₄, nTG52₈₆₇, nTG53₁₄₃₄, nTG54₆₈₀, nTG55₂₉₆, nTG56₇₂₃, nTG57₂₇₀, nTG58₅₀₃, nTG60₃₂₂, nTG61₃₉₀, nTG62₆₉₉, nTG63₄₁₉, nTG64₃₀₃, nTG65₆₉₆, nTG66₁₇₃, nTG67₃₆₉, nTG68₅₆₆, nTG69₆₁₆, nTG70₇₆₂, nTG71₂₃₆, nTG72₅₆₉, nTG73₂₃₂, nTG74₂₇₆, nTG75₃₀₉, nTG76₅₃₄, nTG76₄₂₃, nTG77₃₂₇, nTG78₄₄₄, nTG79₉₂₈, nTG22_{310a}, nTG64_{303a}, nTG71_{236a}, nTG64_{425a}, nTG41_{513a}, nTG₆₇₇, nTG73₃₉₇, nTG7₇₃₂₇, nTG78₄₄₄, nTG79₉₂₈, nTG22_{310a}, nTG64_{303a}, nTG71_{236a}, nTG6_{425a}, nTG41_{513a}, nTG₆₇₇, nTG73₃₉₇, nTG₁₇₈₅.
- The nucleic acid molecule of Claim 1, 4 or 5, wherein said nucleic acid molecule 24. is at least about 75% identical to a nucleic acid molecule selected from the group 15 consisting of nTG2₃₃₉, nTG4₅₂₆, nTG4₁₄₇₈, nTG5₆₅₇, nTG5₁₀₂₉, nTG6₄₂₅, nTG7₄₁₇, nTG8₅₀₇, $nTG9_{718}, nTG10_{441}, nTG11_{428}, nTG13_{282}, nTG15_{304}, nTG16_{284}, nTG17_{690}, nTG18_{313}, nTG19_{718}, nTG10_{441}, nTG11_{428}, nTG13_{428}, nTG15_{441}, nTG10_{441}, nTG1$ $nTG19_{389},\,nTG21_{548},\,nTG22_{310},\,nTG23_{220},\,nTG24_{642},\,nTG25_{381},\,nTG26_{432},\,nTG27_{282},\,nTG$ $nTG28_{466},\,nTG30_{539},\,nTG31_{1233}\,,\,nTG32_{411},\,nTG33_{441},\,nTG34_{491},\,nTG35_{387},\,nTG36_{417},\,n$ $nTG37_{416},\,nTG38_{500},\,nTG40_{321},\,nTG41_{513},\,nTG42_{528},\,nTG43_{375},\,nTG44_{543},\,nTG45_{573},\,nTG$ $nTG46_{1835},\,nTG48_{604},\,nTG48_{549},\,nTG49_{270}\,,\,nTG50_{306},\,nTG51_{804},\,nTG52_{867},\,nTG53_{1434},\,nTG52_{867},\,nTG53_{1434},\,nTG52_{867},\,nTG53_{1434},\,nTG53_{$ $nTG54_{680},\,nTG55_{296},\,nTG56_{723},\,nTG57_{270},\,nTG58_{503},\,nTG60_{322},\,nTG61_{390},\,nTG62_{699},\,nTG54_{680},\,nTG55_{680},\,nTG61_{680},\,nTG$ $nTG63_{419},\ nTG64_{303},\ nTG65_{696},\ nTG66_{173},\ nTG67_{369},\ nTG68_{566},\ nTG69_{616},\ nTG70_{762},$ $nTG71_{236},\,nTG72_{569},\,nTG73_{232},\,nTG74_{276},\,nTG75_{309},\,n\Upsilon G76_{534},\,nTG76_{423},\,nTG77_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{327},\,nTG7_{3$ $nTG78_{444},\,nTG79_{928,}\,nTG22_{310a},\,nTG64_{303a},\,nTG71_{236a},\,nTG6_{425a},\,nTG41_{513a},\,nTG_{867,}\,nTG71_{236a},\,nTG8_{425a},\,$ 25 nTG₁₃₉₇, nTG₁₇₈₅.
 - 25. The nucleic acid molecule of Claim 1, 4 or 5, wherein said nucleic acid molecule is selected from the group consisting of: a nucleic acid molecule selected from the group consisting of nTG2₃₃₉, nTG4₅₂₆, nTG4₁₄₇₈, nTG5₆₅₇, nTG5₁₀₂₉, nTG6₄₂₅, nTG7₄₁₇, nTG8₅₀₇,
 - $30 \quad \text{nTG9}_{718}, \, \text{nTG10}_{441}, \, \text{nTG11}_{428}, \, \text{nTG13}_{282}, \, \text{nTG15}_{304}, \, \text{nTG16}_{284}, \, \text{nTG17}_{690}, \, \text{nTG18}_{313}, \, \text{nTG18}_{313}, \, \text{nTG19}_{11}, \, \text{nTG19}$

 $nTG19_{389}$, $nTG21_{548}$, $nTG22_{310}$, $nTG23_{220}$, $nTG24_{642}$, $nTG25_{381}$, $nTG26_{432}$, $nTG27_{282}$ $nTG28_{466}$, $nTG30_{539}$, $nTG31_{1233}$, $nTG32_{411}$, $nTG33_{441}$, $nTG34_{491}$, $nTG35_{387}$, $nTG36_{417}$, nTG37₄₁₆, nTG38₅₀₀, nTG40₃₂₁, nTG41₅₁₃, nTG42₅₂₈, nTG43₃₇₅, nTG44₅₄₃, nTG45₅₇₃, $nTG46_{1835}$, $nTG48_{604}$, $nTG48_{549}$, $nTG49_{270}$, $nTG50_{306}$, $nTG51_{804}$, $nTG52_{867}$, $nTG53_{1434}$, $nTG54_{680}$, $nTG55_{296}$, $nTG56_{723}$, $nTG57_{270}$, $nTG58_{503}$, $nTG60_{322}$, $nTG61_{390}$, $nTG62_{699}$ $nTG63_{419}$, $nTG64_{303}$, $nTG65_{696}$, $nTG66_{173}$, $nTG67_{369}$, $nTG68_{566}$, $nTG69_{616}$, $nTG70_{762}$ nTG71₂₃₆, nTG72₅₆₉, nTG73₂₃₂, nTG74₂₇₆, nTG75₃₀₉, nTG76₅₃₄, nTG76₄₂₃, nTG77₃₂₇, $nTG78_{444}$, $nTG79_{928}$ $nTG22_{310a}$, $nTG64_{303a}$, $nTG71_{236a}$, $nTG6_{425a}$, $nTG41_{513a}$, nTG_{867} nTG₁₃₉₇, nTG₁₇₈₅; and a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule selected from the group consisting of nTG2₃₃₉, nTG4₅₂₆, nTG4₁₄₇₈, 10 $nTG5_{657}$, $nTG5_{1029}$, $nTG6_{425}$, $nTG7_{417}$, $nTG8_{507}$, $nTG9_{718}$, $nTG10_{441}$, $nTG11_{428}$, $nTG13_{282}$, nTG15304, nTG16284, nTG17690, nTG18313, nTG19389, nTG21548, nTG22310, nTG23220, $nTG24_{642}$, $nTG25_{381}$, $nTG26_{432}$, $nTG27_{282}$, $nTG28_{466}$, $nTG30_{539}$, $nTG31_{1233}$, $nTG32_{411}$, $nTG33_{441}$, $nTG34_{491}$, $nTG35_{387}$, $nTG36_{417}$, $nTG37_{416}$, $nTG38_{500}$, $nTG40_{321}$, $nTG41_{513}$, $nTG42_{528}$, $nTG43_{175}$, $nTG44_{543}$, $nTG45_{573}$, $nTG46_{1835}$, $nTG48_{604}$, $nTG48_{549}$, $nTG49_{270}$, 15 $nTG50_{306}$, $nTG51_{804}$, $nTG52_{867}$, $nTG53_{1434}$, $nTG54_{680}$, $nTG55_{296}$, $nTG56_{723}$, $nTG57_{270}$, $nTG58_{503}$, $nTG60_{322}$, $nTG61_{390}$, $nTG62_{699}$, $nTG63_{419}$, $nTG64_{303}$, $nTG65_{696}$, $nTG66_{173}$, nTG67₃₆₉, nTG68₅₆₆, nTG69₆₁₆, nTG70₇₆₂, nTG71₂₃₆, nTG72₅₆₉, nTG73₂₃₂, nTG74₂₇₆, $nTG75_{309}, nTG76_{534}, nTG76_{423}, nTG77_{327}, nTG78_{444}, nTG79_{928}, nTG22_{310a}, nTG64_{303a}$ nTG71_{236a}, nTG6_{425a}, nTG41_{513a}, nTG₈₆₇, nTG₁₃₉₇, nTG₁₇₈₅. The nucleic acid molecule of Claim 1, 4 or 5, wherein said nucleic acid molecule 26. encodes a protein comprising an amino acid sequence that is at least about 75% identical to an amino acid sequence encoded by a nucleic acid molecule selected from the group consisting of nTG2₁₃₉, nTG4₅₂₆, nTG4₁₄₇₈, nTG5₆₅₇, nTG5₁₀₂₉, nTG6₄₂₅, nTG7₄₁₇, nTG8₅₀₇, $nTG9_{718}$, $nTG10_{441}$, $nTG11_{428}$, $nTG13_{282}$, $nTG15_{304}$, $nTG16_{284}$, $nTG17_{690}$, $nTG18_{313}$, $nTG19_{389}$, $nTG21_{548}$, $nTG22_{310}$, $nTG23_{220}$, $nTG24_{642}$, $nTG25_{381}$, $nTG26_{432}$, $nTG27_{282}$, $nTG28_{466}$, $nTG30_{539}$, $nTG31_{1233}$, $nTG32_{411}$, $nTG33_{441}$, $nTG34_{491}$, $nTG35_{387}$, $nTG36_{417}$, nTG37₄₁₆, nTG38₅₀₀, nTG40₃₂₁, nTG41₅₁₃, nTG42₅₂₈, nTG43₃₇₅, nTG44₅₄₃, nTG45₅₇₃, $nTG46_{1835}$, $nTG48_{604}$, $nTG48_{549}$, $nTG49_{270}$, $nTG50_{306}$, $nTG51_{804}$, $nTG52_{867}$, $nTG53_{1434}$, $nTG54_{680}, nTG55_{296}, nTG56_{723}, nTG57_{270}, nTG58_{503}, nTG60_{322}, nTG61_{390}, nTG62_{699},$ 30

 $nTG63_{419}, nTG64_{303}, nTG65_{696}, nTG66_{173}, nTG67_{369}, nTG68_{566}, nTG69_{616}, nTG70_{762}, \\ nTG71_{236}, nTG72_{569}, nTG73_{232}, nTG74_{276}, nTG75_{309}, nTG76_{534}, nTG76_{423}, nTG77_{327}, \\ nTG78_{444}, nTG79_{928}, nTG22_{310a}, nTG64_{303a}, nTG71_{236a}, nTG6_{425a}, nTG41_{513a}, nTG_{867}, \\ nTG_{1397}, nTG_{1785}.$

- The immunogenic protein of Claim 3, 6 or 7, wherein said protein is selected 5 27. from the group consisting of: a protein comprising an amino acid sequence that is at least about 75% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID 10 NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID 15 NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:133, SEQ ID NO:135, SEQ ID 20 NO:137, SEQ ID NO:140, SEQ ID NO:142, SEQ ID NO:144, SEQ ID NO:266, SEQ ID NO:268, SEQ ID NO:270, SEQ ID NO:272, SEQ ID NO:274, SEQ ID NO:283, SEQ ID NO:293, SEQ ID NO:307, SEQ ID NO:312, and SEQ ID NO:341; and a protein comprising an epitope of said protein having said amino acid sequence.
 - 25 28. The immunogenic protein of Claim 3, 6 or 7, wherein said protein is selected from the group consisting of: an immunogenic protein encoded by a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID

NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEO ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEO ID NO:54, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:50, SEO ID NO:59, SEO ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEO ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEO ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEO ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEO ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEO ID NO:117, SEO ID 10 NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEO ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEO ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:340, and SEQ ID NO:343; and an immunogenic protein encoded by a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule comprising any of said nucleic 15 acid sequences.

29. The immunogenic protein of Claim 3, 6 or 7, wherein said protein is selected from the group consisting of: an immunogenic protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, 20 SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:56, SEQ ID NO:58, SEQ ID 25 NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID 30 NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID

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- 30. The immunogenic protein of Claim 3, 6 or 7, wherein said protein comprises an epitope that elicits an immune response.
- The immunogenic protein of Claim 3, 6 or 7, wherein said protein is selected from the group consisting of: an immunogenic protein encoded by a nucleic acid 10 molecule selected from the group consisting of:nTG2₃₃₉, nTG4₅₂₆, nTG4₁₄₇₈, nTG5₆₅₇, $nTG5_{1029}, nTG6_{425}, nTG7_{417}, nTG8_{507}, nTG9_{718}, nTG10_{441}, nTG11_{428}, nTG13_{282}, nTG15_{304}, nTG10_{441}, nTG11_{428}, nTG13_{441}, nTG11_{441}, nTG11_{$ $nTG16_{284}, nTG17_{690}, nTG18_{313}, nTG19_{389}, nTG21_{548}, nTG22_{310}, nTG23_{220}, nTG24_{642}, nTG19_{389}, nTG21_{548}, nTG22_{310}, nTG23_{320}, nTG24_{642}, nTG21_{548}, nTG22_{548}, nTG22_{548}, nTG23_{548}, nTG$ $nTG25_{381},\,nTG26_{432},\,nTG27_{282},\,nTG28_{466},\,nTG30_{539},\,nTG31_{1233}\,,\,nTG32_{411},\,nTG33_{441},\,n$ $nTG34_{491},\,nTG35_{387},\,nTG36_{417},\,nTG37_{416},\,nTG38_{500},\,nTG40_{321},\,nTG41_{513},\,nTG42_{528},\,nTG38_{500},\,nTG40_{321},\,nTG41_{513},\,nTG42_{528},\,nTG40_{321},\,nTG$ $nTG43_{375},\,nTG44_{543},\,nTG45_{573},\,nTG46_{1835},\,nTG48_{604},\,nTG48_{549},\,nTG49_{270}\;,\,nTG50_{306},\,nTG48_{549},\,nTG49_{549},\,nTG49_{549},\,nTG49_{549},\,nTG50_{549},\,n$ $nTG51_{804}, nTG52_{867}, nTG53_{1434}, nTG54_{680}, nTG55_{296}, nTG56_{723}, nTG57_{270}, nTG58_{503}, nTG51_{804}, nT$ $nTG60_{322},\,nTG61_{390},\,nTG62_{699},\,nTG63_{419},\,nTG64_{303},\,nTG65_{696},\,nTG66_{173},\,nTG67_{369},\,nTG60_{322},\,nTG61_{390},\,nTG60_{322},\,nTG$ $nTG68_{566}, nTG69_{616}, nTG70_{762}, nTG71_{236}, nTG72_{569}, nTG73_{232}, nTG74_{276}, nTG75_{309}, nTG70_{762}, nTG$ $nTG76_{534},\,nTG76_{423},\,nTG77_{327},\,nTG78_{444},\,nTG79_{928},\,nTG22_{310a},\,nTG64_{303a},\,nTG71_{236a},\,nTG71$ 20 nTG6_{425a}, nTG41_{513a}, nTG₈₆₇, nTG₁₃₉₇, nTG₁₇₈₅; and an immunogenic protein encoded by a nucleic acid molecule comprising an allelic variant of any of said nucleic acid
 - 32. The invention of Claim 8 or 9, wherein said composition further comprises a component selected from the group consisting of an excipient, an adjuvant, and a carrier.
 - 33. The invention of Claim 8 or 9, wherein said compound is selected from the group consisting of a genetic vaccine, a recombinant virus vaccine and a recombinant cell vaccine.
 - 34. The invention of Claim 8 or 9, wherein said compound comprises a recombinant molecule.

molecules.

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- 35. The invention of Claim 8 or 9, wherein said compound is selected from the group consisting of a recombinant virus genome and a recombinant plasmid.
- 36. The invention of Claim 8 or 9, wherein said composition is administered by a method selected from the group consisting of injection, oral administration, inhalation,
- nasal administration, intraocular administration, anal administration, topical administration, particle bombardment, and intradermal scarification.
 - 37. The invention of Claim 8 or 9, wherein said composition is administered by a method selected from the group consisting of intradermal injection and intramuscular injection.
- 10 38. The invention of Claim 8 or 9, wherein said composition is administered mucosally.
 - 39. A method to detect parasite cysts or oocysts in feces, said method comprising:
 - 1. contacting a sample of feces with a solid support capable of binding oocysts;
 - 2. allowing the sample to dry onto the solid support;
- 15 3. washing the sample on the solid support with an aqueous wash solution;
 - 4. adding an aqueous elution solution to the sample and eluting DNA from the sample into the aqueous elution solution by heating;
 - 5. PCR amplifying oocyst-specific DNA with primers specific to the oocyst being detected; and
- 6. detecting the presence of a PCR amplification product resulting from amplification of oocyst-specific DNA in the sample wherein the presence of said product indicates the presence of cysts or oocysts in said feces.
 - 40. A method according to Claim 39, wherein the sample of feces is solubilized in an aqueous solution before contacting the sample with a solid support capable of binding oocysts.
 - 41. A method according to Claim 39, wherein the aqueous wash solution comprises distilled water.
 - 42. A method according to Claim 39, wherein the aqueous elution solution comprises distilled water.

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- 43. A method according to Claim 39, wherein the heating step comprises heating to approximately 95° C.
- 44. A method according to Claim 39, wherein the solid support capable of binding oocysts comprises paper.
- 5 45. A method according to Claim 39, wherein the solid support comprises one or more compounds capable of binding inhibitors of PCR amplification.
 - 46. The method of Claim 39, wherein the parasite oocysts are enteric apicomplexa oocysts.
- 47. The method of Claim 46 wherein the enteric apicomplexa oocysts are selected from the group consisting of *Cryptosporidium* oocysts and *Toxoplasma* oocysts.

SEQUENCE LISTING

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_	_	-	_				_	-		cct Pro	-		-	_	-	627
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		-	_	_						gac Asp					-	819
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Ser Pro Ala Leu Val Pro Pro Ala Glu Thr Glu Glu Gly Thr Ala Ala 290 295 300

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Arg Pro Gln Glu Val Leu Thr Arg His Thr Trp Gln Asp Met Glu Arg 325 330 335

Thr Glu Asp Leu Arg Lys Asn Asp Val Pro Ala Ala Val Ala Asn Ser 340 345 350

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gcc gag atg ccc aag tta tca gat ata ccc aag atg gct gag atg ccc 192

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					tta Leu											336
					cct Pro										-	384
					gat Asp									_		432
					ttt Phe 150									_		480
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Lys Leu Ser Asp Ile Pro Lys Met Ala Glu Met Pro Lys Leu Ser Asp

65 70 75 80

ata ccc aag atg gct gag atg ccc aag ttt tca gat ata ccc aag atg 288

Ile Pro Lys Met Ala Glu Met Pro Lys Phe Ser Asp Ile Pro Lys Met

85 90 95

gct gag atg cca aag tta tca gat atg ccc aga atg gct gac att cca 336 Ala Glu Met Pro Lys Leu Ser Asp Met Pro Arg Met Ala Asp Ile Pro 100 105 110

cag ttt cca gag atg cct agg atg gtt gac atg cct cag ttt cca gaa 384 Gln Phe Pro Glu Met Pro Arg Met Val Asp Met Pro Gln Phe Pro Glu 115 120 125

atc ccc agg atg gct gat atg cgg aga ttt ccg gag atg tcc aag ata 432 .

Ile Prc_Arg Met Ala Asp Met Arg Arg Phe Pro Glu Met Ser Lys Ile
130 135 140

gct gac atg cca aag ttt cca gac atg cca aac gtc act gag atg cca 480 Ala Asp Met Pro Lys Phe Pro Asp Met Pro Asn Val Thr Glu Met Pro 145 150 155 160

aag ctt gca gat ttg cca agg ctt gct gac atg ccc agt att gcc gac 528

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Lys	Leu	Ala	Asp	Leu 165	Pro	Arg	Leu	Ala	Asp 170	Met	Pro	Ser	Ile	Ala 175	Asp	
atg Met	ccc Pro	cgg Arg	ctc Leu 180	tca Ser	gac Asp	atg Met	ccc Pro	agt Ser 185	att Ile	gca Ala	gac Asp	atg Met	ccc Pro 190	cgg Arg	ctc Leu	576
tca Ser	gac Asp	ata Ile 195	ccc Pro	agt Ser	att Ile	gcc Ala	gac Asp 200	atg Met	ccc Pro	cgg Arg	ctc Leu	tca Ser 205	gac Asp	atg Met	ccc Pro	624
agt Ser	att Ile 210	gcc Ala	gac Asp	atg Met	ccg Pro	aaa Lys 215	ttc Phe	tct Ser	agt Ser	aac Asn	cga Arg 220	gtt Val	cat His	Gly	caa Gln	672
agt Ser 225	tac Tyr	cat His	att Ile	ctg Leu	gcg Ala 230	ata Ile	tgg Trp	aca Thr	ccg Pro	tcc Ser 235	ctt Leu	tcc Ser	gga Gly	ctc Leu	aag Lys 240	720
gag Glu	ttt Phe	ttt Phe	acc Thr	ccg Pro 245	ctc Leu	tct Ser	gac Asp	cta Leu	atc Ile 250	aag Lys	cca Pro	gaa Glu	gct Ala	gct Ala 255	tcc Ser	768
ctg Leu	aca Thr	agc Ser	ctg Leu 260	Ala	aag Lys	cca Pro	tct Ser	gga Gly 265	Val	ttt Phe	ctg Leu	aga Arg	acc Thr 270	ctg Leu	ctg Leu	816
gct Ala		tgag	aaa	atgt	atat	tg a	caaa	tggc	t gt	atct	ccat	agt	tata	gtg		869
agg	aato	jtat	tgac	ttat	tc c	gagg	acto	t at	actg	aacc	: cgc	:ggca	tac	gagg	aaactg	929
aca	agtt	ggt	gato	gtgcc	jtt t	ctga	tctt	:c c	ecgaa	aaga	aaa	aaaa	atg	acco	tcttaa	989
aaa	aaaa	aaaa	aaaa	aaaa	aaa a	aaaa	aaaa	aa aa	aaaa	ıaaaa	ì					1029
	10>															

<212> PRT

<213> Toxoplasma gondii

Glu Met Ser Ala Pro Asp Arg Gln Thr Gly Lys Leu Ser Asp Leu Pro 10 5 1

Pro Phe Ala Glu Leu Pro Gln Leu Ala Glu Ile Pro Lys Leu Ser Glu 25 20

Leu Pro Lys Ile Ala Asp Met Pro Lys Phe Ser Asp Met Pro Lys Met 35 40 Ala Glu Met Pro Lys Leu Ser Asp Ile Pro Lys Met Ala Glu Met Pro 50 Lys Leu Ser Asp Ile Pro Lys Met Ala Glu Met Pro Lys Leu Ser Asp 70 Ile Pro Lys Met Ala Glu Met Pro Lys Phe Ser Asp Ile Pro Lys Met Ala Glu Met Pro Lys Leu Ser Asp Met Pro Arg Met Ala Asp Ile Pro 100 105 Gln Phe Pro Glu Met Pro Arg Met Val Asp Met Pro Gln Phe Pro Glu 120 Ile Pro Arg Met Ala Asp Met Arg Arg Phe Pro Glu Met Ser Lys Ile 130 135 140 Ala Asp Met Pro Lys Phe Pro Asp Met Pro Asn Val Thr Glu Met Pro 145 150 155 Lys Leu Ala Asp Leu Pro Arg Leu Ala Asp Met Pro Ser Ile Ala Asp 165 170 Met Pro Arg Leu Ser Asp Met Pro Ser Ile Ala Asp Met Pro Arg Leu Ser Asp Ile Pro Ser Ile Ala Asp Met Pro Arg Leu Ser Asp Met Pro 200 Ser Ile Ala Asp Met Pro Lys Phe Ser Ser Asn Arg Val His Gly Gln 210 215 220 Ser Tyr His Ile Leu Ala Ile Trp Thr Pro Ser Leu Ser Gly Leu Lys 225 230 240 Glu Phe Phe Thr Pro Leu Ser Asp Leu Ile Lys Pro Glu Ala Ala Ser 245 250 Leu Thr Ser Leu Ala Lys Pro Ser Gly Val Phe Leu Arg Thr Leu Leu 265

Ala

<210: <211: <212: <213:	> 42 > DN > To	A	asma	gone	dii											
<220 <221 <222	> CD		423)													
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agt Ser	ctg Leu	gtt Val	ttg Leu 20	ccc Pro	tcc Ser	aga Arg	G] À	gag Glu 25	gaa Glu	gag Glu	gcg Ala	aga Arg	gag Glu 30	gag Glu	acg Thr	96
tct Ser	gca Ala	acg Thr 35	cgc Arg	cag Gln	atg Met	ccg Pro	acg Thr 40	ctt Leu	ctc Leu	tct Ser	tcg Ser	ccg Pro 45	agg Arg	cct Pro	cca Pro	144
ctc Leu	gcg Ala 50	ctg Leu	G] À ddd	ttg Leu	gga Gly	gac Asp 55	aag Lys	tct Ser	ccc Pro	tgc Cys	gga Gly 60	gag Glu	tgg Trp	gtg Val	tcg Ser	192
ccg Pro 65	aat Asn	gac Asp	atg Met	gtt Val	tct Ser 70	gcg Ala	ttg Leu	tcc Ser	ctc Leu	tgg Trp 75	gaa Glu	gca Ala	ggc	gag Glu	gct Ala 80	240
tgg Trp	cag Gln	ttc Phe	: aag : Lys	aca Thr 85	Ala	aaa Lys	att Ile	ctt	gac Asp 90	Ser	ttc Phe	gaa Glu	ggg ggg	gag Glu 95	acc Thr	288
cca Pro	gaa Glu	a ggg	g gag y Glu 100	Gly	tgc Cys	: ggc	gca Ala	Glr 105	n Glu	aga Arç	ago J Aro	, aca , Thr	gcc Ala 110	i Alc	tgc Cys	336
aág Lys	g cto	g gto u Va 11	l Ar	a cto g Lei	e ccq	g gto o Val	aac Asr 120	ı Va	g gaq 1 Glu	g ggg ı Gl	g cgo	g tco g Sei 125	111.	a aac r Ly:	g gtg s Val	384
tg: Tr	g ag p Se 13	r Le	g gc u Al	t ct a Le	t ct u Le	t tc u Se 13	r Se	t ct r Le	g cg u Ar	t ct g Le	g aa u Ly 14	s II	c cg e			425

<210> 14 <211> 141 <212> PRT <213> Toxoplasma gondii <400> 14 Arg Gly Ile Pro Asp Gln Arg Ser Ser Arg Ser His Thr Gly Val Glu 10 Ser Leu Val Leu Pro Ser Arg Gly Glu Glu Glu Ala Arg Glu Glu Thr 25 Ser Ala Thr Arg Gln Met Pro Thr Leu Leu Ser Ser Pro Arg Pro Pro 40 Leu Ala Leu Gly Leu Gly Asp Lys Ser Pro Cys Gly Glu Trp Val Ser Pro Asn Asp Met Val Ser Ala Leu Ser Leu Trp Glu Ala Gly Glu Ala 65 70 75 Trp Gln Phe Lys Thr Ala Lys Ile Leu Asp Ser Phe Glu Gly Glu Thr Pro Glu Gly Glu Gly Cys Gly Ala Gln Glu Arg Arg Thr Ala Ala Cys 100 105 Lys Leu Val Arg Leu Pro Val Asn Val Glu Gly Arg Ser Thr Lys Val 115 120 Trp Ser Leu Ala Leu Leu Ser Ser Leu Arg Leu Lys Ile 135 <210> 15 <211> 417 <212> DNA <213> Toxoplasma gondii <220> <221> CDS <222> (1)..(417) ege gge ett tee gat gae gee tet eae geg gag aee eet tea eeg ete

Arg Gly Leu Ser Asp Ala Ser His Ala Glu Thr Pro Ser Pro Leu

10

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acg Thr	ccc Pro	tcg Ser	agg Arg 20	gtg Val	gac Asp	agc Ser	ttc Phe	tca Ser 25	gac Asp	gga Gly	gtt Val	gag Glu	aga Arg 30	aca Thr	cgc Arg	96
aga Arg	agc Ser	tct Ser 35	ccg Pro	cga Arg	gtc Val	gag Glu	gag Glu 40	cac His	cag Gln	acg Thr	agc Ser	tcg Ser 45	aga Arg	gag Glu	gaa Glu	144
aaa Lys	gct Ala 50	gcg Ala	aca Thr	gag Glu	cgc Arg	gtt Val 55	cca Pro	aaa Lys	ctg Leu	tct Ser	cgt Arg 60	ctc Leu	ccc Pro	tcg Ser	ctc Leu	192
cga Arg 65	gct Ala	cct Pro	cta Leu	cgc Arg	agc Ser 70	acg Thr	gac Asp	cga Arg	cgc Arg	gcc Ala 75	tcg Ser	ccg Pro	cct Pro	cgt Arg	cgg Arg 80	240
ctg Leu	tcg Ser	caa Gln	ctt Leu	ctt Leu 85	cgc Arg	tgc Cys	tgc Cys	aca Thr	acc Thr 90	tcg Ser	aga Arg	ttc Phe	gcg Ala	agc Ser 95	aaa Lys	288
gga Gly	acg Thr	gcg	tat Tyr 100	Pro	gac Asp	gag Glu	gag Glu	tgg Trp 105	GLY	cat His	aga Arg	gtc Val	cga Arg 110	AT G	cag Gln	336
aga Arg	aca g Thr	gaa Glu	ı Glu	g act 1 Thr	gtc Val	tcc Ser	tct Ser 120	Leu	acq Thr	acg Thr	g aaq Lys	g cgc s Arg 125	Lec	cto Lei	act Thr	384
cg: Ar	a agt g Se: 13	r Pr	t aat o Ası	t tog	g caq r Glr	g act n Thi 13!	r Ala	c tto a Phe	e ccq	g cgq	3					417
<2 <2	10> 11> 12> 13>	139 PRT	oplas	ma g	ondi	i										
< 4 A1	100> :g G]	16 y Le	eu Se	er As	sp As	(A q	la Se	er Hi	s Al	.a Gl .0	lu Th	nr Pr	o Se	er Pr	o Leu 15	
Tl	nr Pi	ro S		rg Va 20	al As	sp Se	er Pi	ne Se	er As 25	sp G	ly Va	al G	lu Ai	rg T1 30	nr Arg	
A	rg S	er S	er P 35	ro A	rg V	al G	lu G	lu H 40	is G	ln T	hr S	er S	er A: 45	rg G	lu Glu	

Lys Ala Ala Thr Glu Arg Val Pro Lys Leu Ser Arg Leu Pro Ser Leu 50 55 Arg Ala Pro Leu Arg Ser Thr Asp Arg Ala Ser Pro Pro Arg Arg 65 70 75 Leu Ser Gln Leu Leu Arg Cys Cys Thr Thr Ser Arg Phe Ala Ser Lys 85 Gly Thr Ala Tyr Pro Asp Glu Glu Trp Gly His Arg Val Arg Ala Gln 100 105 Arg Thr Glu Glu Thr Val Ser Ser Leu Thr Thr Lys Arg Leu Leu Thr 120 125 Arg Ser Pro Asn Ser Gln Thr Ala Phe Pro Arg 135 <210> 17 <211> 507 <212> DNA <213> Toxoplasma gondii <220> <221> CDS <222> (1)..(153) <400> 17 ggc agg gga agt gga cga cac ccg tcg ctg agc ttt cgc ctg gag tgg 48 Gly Arg Gly Ser Gly Arg His Pro Ser Leu Ser Phe Arg Leu Glu Trp aga cat cta cct gtg agt gaa cca ggc gtt ctg ctt tcg ccg ctc ctt Arg His Leu Pro Val Ser Glu Pro Gly Val Leu Leu Ser Pro Leu Leu 20 25 30 tgc agg cca gag gac aat gat aca aat ata.agt gac act ctt ctc ttc Cys Arg Pro Glu Asp Asn Asp Thr Asn Ile Ser Asp Thr Leu Leu Phe 35 40 gat atc ggt taactgacaa agaaccacag cggagttaaa atagcagcgt 193 Asp Ile Gly 50

ttgcagttca acgcatgcac aaactgctta actcccacat gcttgccttt gagagacgcg 253

acagcacatc gttcgagctt gcacgcagcg aagacatcta gacagcaatt aggagatgcc 313 tgccgaattt gtatgtaagg cgcaaacgtc tcctcggtgc gaatcacaat tacgcacatt 373 tqcccqqact tacatctqtc ttctactqqq qtctttcctt qtcaaaccqt qccqctqcaa 433 ctccaaacta gctcgttagt gagatgctgg caaggttttg acaagaatcg agttctgcga 493 507 ctgcatcgtg gtcg <210> 18 <211> 51 <212> PRT <213> Toxoplasma gondii <400> 18 Gly Arg Gly Ser Gly Arg His Pro Ser Leu Ser Phe Arg Leu Glu Trp 10 Arg His Leu Pro Val Ser Glu Pro Gly Val Leu Leu Ser Pro Leu Leu 25 Cys Arg Pro Glu Asp Asn Asp Thr Asn Ile Ser Asp Thr Leu Leu Phe 45 35 Asp Ile Gly 50 <210> 19 <211> 718 <212> DNA <213> Toxoplasma gondii <220> <221> CDS <222> (1)..(297) <400> 19 gaa ttc cga ctg aat gac tac ctc ttt cag gtg cca gag ggt ccc ccc Glu Phe Arg Leu Asn Asp Tyr Leu Phe Gln Val Pro Glu Gly Pro Pro 15 10 1 5 gcg aga agc cat ggg ttc gac aga aga cga gca gca gcg agc aaa aac Ala Arg Ser His Gly Phe Asp Arg Arg Arg Ala Ala Ala Ser Lys Asn 30 25 20

			gaa									_	-	-		144
Ala	Thr	Glu 35	Glu	Thr	Arg	Arg	Leu 40	Ala	Gly	Lys	Glu	Thr 45	Pro	Pro	His	
			ccg Pro												• -	192
			gaa Glu												_	240
-		_	gat Asp	-			-					-				288
	gga Gly	_	tgaa	aaaa	igc c	gaag	gatga	c aç	ıgcċa	ıgagt	: aag	gacga	ıgga			337
ggtç	jcago	gac a	aagga	itgtc	t ct	tatt	cacc	gaç	tctc	gtt	aacc	agcç	jtt ç	gtct	tatca	397
agaç	gtgc	ag g	gacac	agat	gaç	jacat	ccgg	tto	gtcc	aaa	gaco	agtt	gg a	gcac	tcgag	457
agaç	gcaa	aga d	cagaa	gctg	ıa go	gtto	gcga	caç	acat	cca	gctg	cctc	cg c	gggc	gttgt	517
tcac	tgaç	gga d	ettgg	gtcgg	a aa	gggg	gagag	aaa	cata	gaa	acga	agaa	ica c	caag	acctg	577
gaag	jaggt	gc a	agatt	cctc	t to	iggca	ctcg	caç	gaga	cgc	cttc	gtca	igt t	tttt	ttgtt	637
cact	caac	cgg a	actct	gtcg	jt ca	acgaç	ggaa	cto	agac	aga	gaco	tcaa	igg a	agaca	gagga	697
acgo	caacç	gca (cgtcc	ggaat	t c											718

<210> 20

<211> 99

<212> PRT

<213> Toxoplasma gondii

<400> 20

Glu Phe Arg Leu Asn Asp Tyr Leu Phe Gln Val Pro Glu Gly Pro Pro 1 5 10 15

Ala Arg Ser His Gly Phe Asp Arg Arg Arg Ala Ala Ser Lys Asn 20 25 30

Ala Thr Glu Glu Thr Arg Arg Leu Ala Gly Lys Glu Thr Pro Pro His 35 40 45

Arg Glu Ala Pro Glu Lys Thr Thr Arg Gly Glu Glu Asp Arg Gln Glu 55 50 Ser Glu Arg Glu Arg Arg Arg Ala Gly Val Met Asp Lys Lys Asn Gln 75 70 Asp Leu Asp Asp Glu Thr Arg Arg Gly Thr Ala Glu Glu Glu Arg 90 85 Asn Gly Asp <210> 21 <211> 441 <212> DNA <213> Toxoplasma gondii <220> <221> CDS <222> (1)..(441) <400> 21 egg ate gee teg gea ett eet eat tat eeg teg eat ggg eat tte etg Arg Ile Ala Ser Ala Leu Pro His Tyr Pro Ser His Gly His Phe Leu 5 gaa gag gaa caa att ttg ctg ttg gat tgg cag tat caa ctt ggg caa 96 Glu Glu Glu Gln Ile Leu Leu Asp Trp Gln Tyr Gln Leu Gly Gln 25 20 cga ggc atg gag tcc ggt gta ccc ccc tgc gtg cag cat ggg gat gcg 144 Arg Gly Met Glu Ser Gly Val Pro Pro Cys Val Gln His Gly Asp Ala 45 40 35 acg aga agt ttg act tca ccg aaa agg gat gtc agt cat gac ggt cac 192 Thr Arg Ser Leu Thr Ser Pro Lys Arg Asp Val Ser His Asp Gly His 55 caa gga aac agc gga aca aac gca gat gaa gcc ggc caa ggg gcc atg Gln Gly Asn Ser Gly Thr Asn Ala Asp Glu Ala Gly Gln Gly Ala Met 75 70 65 gca ggc cga gga aag tgc gag tgg agc cgc acc acc ggt gcc aac gta Ala Gly Arg Gly Lys Cys Glu Trp Ser Arg Thr Thr Gly Ala Asn Val

85

90

ggg tcg tcg tca tgt gtg gtt gat gcg tgt ttg gcg tct gcg ggt aga Gly Ser Ser Cys Val Val Asp Ala Cys Leu Ala Ser Ala Gly Arg 100 cat cag gcg gcg agc atg cgt ccg ttt gca cga gat gga ttc ggc gag 384 His Gln Ala Ala Ser Met Arg Pro Phe Ala Arg Asp Gly Phe Gly Glu 120 125 tot act gog gag aac aga coc ogt ogg gac ggc gga otg coa ogt tot 432 Ser Thr Ala Glu Asn Arg Pro Arg Arg Asp Gly Gly Leu Pro Arg Ser 130 135 ctt gga tcg 441 Leu Gly Ser 145 <210> 22 <211> 147 <212> PRT <213> Toxoplasma gondii <400> 22 Arg Ile Ala Ser Ala Leu Pro His Tyr Pro Ser His Gly His Phe Leu 10 Glu Glu Glu Gln Ile Leu Leu Asp Trp Gln Tyr Gln Leu Gly Gln 20 25 Arg Gly Met Glu Ser Gly Val Pro Pro Cys Val Gln His Gly Asp Ala 40 Thr Arg Ser Leu Thr Ser Pro Lys Arg Asp Val Ser His Asp Gly His 50 55 60 Gln Gly Asn Ser Gly Thr Asn Ala Asp Glu Ala Gly Gln Gly Ala Met 70 65 Ala Gly Arg Gly Lys Cys Glu Trp Ser Arg Thr Thr Gly Ala Asn Val 85 90 Gly Ser Ser Cys Val Val Asp Ala Cys Leu Ala Ser Ala Gly Arg 100 105 His Gln Ala Ala Ser Met Arg Pro Phe Ala Arg Asp Gly Phe Gly Glu

Ser Thr Ala Glu Asn Arg Pro Arg Asp Gly Gly Leu Pro Arg Ser

120

130 135 140

Leu Gly Ser 145

<210> 23

<211> 428

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(426)

<400> 23

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Arg Arg Arg Gln Arg Ala Asp Pro Ser Asp Trp Glu Gly Cys Glu Asn
1 5 10

gtg gaa aag gat cat ttc ggg agt cgc gag agg cac tcg aat qgg gaa 96 Val Glu Lys Asp His Phe Gly Ser Arg Glu Arg His Ser Asn Gly Glu 20 25 30

gag ttc aag aca cag gga aac gtt ggt cga ggt tca ctg agg cag gua 144 Glu Phe Lys Thr Gln Gly Asn Val Gly Arg Gly Ser Leu Arg Gln Glu 35 40 45

ccc ttt acc gat gga gtg tac cac gac agg cag cag cgc ttc tcg qu; 192 Pro Phe Thr Asp Gly Val Tyr His Asp Arg Gln Gln Arg Phe Ser Glu 50 55 60

aaa gaa oot gog aag oog atg tto act too oto gog gat oog ago gtg 240 Lys Glu Pro Ala Lys Pro Met Phe Thr Ser Leu Ala Asp Pro Ser Val 65 70 75 80

agg aga cat ttt aag gag gaa gaa gaa cga cgg aaa ttc cag qua aaa = 288 Arg Arg His Phe Lys Glu Glu Glu Glu Arg Arg Lys Phe Gln Glu Lys 85 90 95

gca gaa gag gag atc ttg cgc ctt ctc aaa cgc gca gct gag tgc ag: 356 Ala Glu Glu Glu Ile Leu Arg Leu Leu Lys Arg Ala Ala Glu Cys Ser 100 105 110

gag gaa gat ttg aaa agg gaa gaa cgc tcc gaa aag gct acc gaa aug 384 Glu Glu Asp Leu Lys Arg Glu Glu Arg Ser Glu Lys Ala Thr Glu Lys 115 120 125

ggg tcc cgt ctc ttc tct gga gag gag gtg cga ttc ttt ccg cc Gly Ser Arg Leu Phe Ser Gly Glu Glu Val Arg Phe Phe Pro 130 135 140

428

. <210> 24

<211> 142

<212> PRT

<213> Toxoplasma gondii

<400> 24

Arg Arg Gln Arg Ala Asp Pro Ser Asp Trp Glu Gly Cys Glu Asn
1 5 10 15

Val Glu Lys Asp His Phe Gly Ser Arg Glu Arg His Ser Asn Gly Glu
20 25 30

Glu Phe Lys Thr Gln Gly Asn Val Gly Arg Gly Ser Leu Arg Gln Glu
35 40 45

Pro Phe Thr Asp Gly Val Tyr His Asp Arg Gln Gin Arg Phe Ser Glu 50 55 60

Lys Glu Pro Ala Lys Pro Met Phe Thr Ser Leu Ala Asp Pro Ser Val 65 70 75 80

Arg Arg His Phe Lys Glu Glu Glu Glu Arg Arg Lys Phe Gln Glu Lys
85 90 95

Ala Glu Glu Ile Leu Arg Leu Leu Lys Arg Ala Ala Glu Cys Ser 100 105 110

Glu Glu Asp Leu Lys Arg Glu Glu Arg Ser Glu Lys Ala Thr Glu Lys 115 120 125

Gly Ser Arg Leu Phe Ser Gly Glu Glu Val Arg Phe Phe Pro 130 135 140

<210> 25

<211> 282

<212> DNA

<213> Toxoplasma gondii

<400> 25

cgcgacccgc tgccagtgtt ttgagtctaa ccgccgtatg tcgcggattc cacgtgqaaa 60

acgacggacc gtcaagacgc ccgagagtgc cgcaatttca cggaccgttc gttcgattcc 120

acc	aaca	cct	tacç	ctac	gcg d	ttgo	tago	ja aa	acaca	acato	g cga	acggo	cggc	tgg	gcctgc	180
tcg	cgga	itct	atco	gtac	aa t	ggga	igaat	c gt	ctga	atgto	tec	cacto	gtcc	cgct	accgca	240
cag	gtco	tct	acag	gcca	ca c	cggt	agac	a gt	gago	ggcg	gc					282
<210> 26 <211> 304 <212> DNA <213> Toxoplasma gondii																
<220> <221> CDS																
<221> CDS <222> (1)(303)																
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Arg 1	Thr	Gly	Thr	Gly 5	Pro	Lys	Arg	Ser	Ser 10	Ser	Lys	Pro	Thr	Ser 15	Thr	
tgg	gtc	cga	ttg	tta -	gtc	cat	act	gaa	aca	aca	atg	gaa	aac	gaa	ttg '	96
Trp	Val	Arg	Leu 20	Leu	Val	His	Thr	Glu 25	Thr	Thr	Met	Glu	Asn 30	Glu	Leu	
atg	aac	caa	gta	agc	gac	ctc	tcg	aat	gag	gct	tgg	caa	aag	aaa	gaa	144
Met	Asn	35	Val	Ser	Asp	Leu	Ser 40	Asn	Glu	Ala	Trp	Gln 45	Lys	Lys	Glu	
										cct						192
Leu	Pro 50	Val	Leu	His	Lys	Trp 55	Thr	Asn	Ser	Pro	Glu 60	His	Ser	Leu	Leu	
										aag						240
Thr 65	Ser	Glu	Asp	Arg	Glu 70	Asn	Ser	Leu	Ser	Lys 75	Pro	Thr	Ala	Asp	Ser 80	
cca	gac	agc	ttc	cgg	tat	ggc	aca	cgc	aga	caa	agt	cac	gca	aaa	gat	288
rro	Asp	ser	Phe	Arg 85	Tyr	Gly	Thr	Arg	Arg 90	Gln	Ser	His	Ala	Lys 95	Asp	
		tcc			g											304
Leu	rne	Ser	100	Pro												

<210> 27

<211> 101

<212> PRT

<213> Toxoplasma gondii

<400> 27

Arg Thr Gly Thr Gly Pro Lys Arg Ser Ser Ser Lys Pro Thr Ser Thr 1 5 10 15

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Met Asn Gln Val Ser Asp Leu Ser Asn Glu Ala Trp Gln Lys Lys Glu . 35 40 45

Leu Pro Val Leu His Lys Trp Thr Asn Ser Pro Glu His Ser Leu Leu 50 55 60

Thr Ser Glu Asp Arg Glu Asn Ser Leu Ser Lys Pro Thr Ala Asp Ser 65 70 75 80

Pro Asp Ser Phe Arg Tyr Gly Thr Arg Arg Gln Ser His Ala Lys Asp 85 90 95

Leu Phe Ser Asp Pro 100

<210> 28

<211> 284

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(282)

<400> 28

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Pro Asp Phe Leu Met Ser Glu Asp Ala Cys Leu Val Arg Phe Val Arg
1 5 10 15

cac gcg tcg gcc aca cac gcg tat aca cgc agg gca agt gcg agg acg 96
His Ala Ser Ala Thr His Ala Tyr Thr Arg Arg Ala Ser Ala Arg Thr
20 25 30

gta aag ccg ctc aaa ggc caa gga gac aaa gaa cag ggt gcg aca gga 144 Val Lys Pro Leu Lys Gly Gln Gly Asp Lys Glu Gln Gly Ala Thr Gly 35 40 45

aga aat gtt gag gca ata aag aag gaa acc cct ctg aga cgg gaa gcg Arg Asn Val Glu Ala Ile Lys Lys Glu Thr Pro Leu Arg Arg Glu Ala aga gaa aac gcg ttt ttt tcg acg ttt tcc ccc gac aga gcg agc gcc Arg Glu Asn Ala Phe Phe Ser Thr Phe Ser Pro Asp Arg Ala Ser Ala 75 70 65 tee tgt etc ege att cac geg tgt gee geg gea gag gaa eec gg 284 Ser Cys Leu Arg Ile His Ala Cys Ala Ala Ala Glu Glu Pro 85 <210> 29 <211> 94 <212> PRT <213> Toxoplasma gondii <400> 29 Pro Asp Phe Leu Met Ser Glu Asp Ala Cys Leu Val Arg Phe Val Arg 15 10 5 His Ala Ser Ala Thr His Ala Tyr Thr Arg Arg Ala Ser Ala Arg Thr 25 20 Val Lys Pro Leu Lys Gly Gln Gly Asp Lys Glu Gln Gly Ala Thr Gly 45 40 35 Arg Asn Val Glu Ala Ile Lys Lys Glu Thr Pro Leu Arg Arg Glu Ala 55 50 Arg Glu Asn Ala Phe Phe Ser Thr Phe Ser Pro Asp Arg Ala Ser Ala 75 70 Ser Cys Leu Arg Ile His Ala Cys Ala Ala Ala Glu Glu Pro 85 <210> 30 <211> 690 <212> DNA <213> Toxoplasma gondii <220>

<221> CDS

<222> (1)..(690)

<400> 30 cga cgt ccc tac cac tat gaa atg ttg gac atc ccg agc atc cgg cgt Arg Arg Pro Tyr His Tyr Glu Met Leu Asp Ile Pro Ser Ile Arg Arg 1 15 gtg gag ttg cca ggt gcg cag gtc cgt atg cca atg gcc aaa gag ctc 96 Val Glu Leu Pro Gly Ala Gln Val Arg Met Pro Met Ala Lys Glu Leu gta cgc gat tgg ggt tct gtc gtc cag cag cag acg act tct gat tct 144 Val Arg Asp Trp Gly Ser Val Val Gln Gln Gln Thr Thr Ser Asp Ser 35 40 tct agt gac aca cca gct acc cgc agt cgc tct gct gaa gca ctc tgt 192 Ser Ser Asp Thr Pro Ala Thr Arg Ser Arg Ser Ala Glu Ala Leu Cys 50 gto ttt tog acg cot tgt aca gca gac ago gac caa cgt atg aaa qqc 240 Val Phe Ser Thr Pro Cys Thr Ala Asp Ser Asp Gln Arg Met Lys Gly 70 75 cgc cat tac cca cag tca tat cat acg ccg agg gac agc gcc acc aaa 288 Arg His Tyr Pro Gln Ser Tyr His Thr Pro Arg Asp Ser Ala Thr Lys 85 90 95 aga gaa aaa cct ctc aaa agt aca ttt atc tgg ggc act aca gtg gaa 336 Arg Glu Lys Pro Leu Lys Ser Thr Phe Ile Trp Gly Thr Thr Val Glu 100 105 gac aga aac cac ccc atc agc cca gac ccg ttc tca agg ctg cag gga 384 Asp Arg Asn His Pro Ile Ser Pro Asp Pro Phe Ser Arg Leu Gln Gly 115 120 tgt ggc cag acc ctc cag gac gag ctc cca tca gct cgc act aga ccg 432 Cys Gly Gln Thr Leu Gln Asp Glu Leu Pro Ser Ala Arg Thr Arg Pro 135 gga tgg gcc gca ttg gac tcc cgc ctg aaa aac aag gac ccq cag att 480 Gly Trp Ala Ala Leu Asp Ser Arg Leu Lys Asn Lys Asp Pro Gln Ile 145 150 155 160 age gea gga gae gaa gee geg aag gte gae gae aeg tea geg gaa eet 528 Ser Ala Gly Asp Glu Ala Ala Lys Val Asp Asp Thr Ser Ala Glu Pro 165 170 175 tgc ctg gga acg gta ccg tcc ttt tgt cgg ctt gta aca agt cac gac Cys Leu Gly Thr Val Pro Ser Phe Cys Arg Leu Val Thr Ser His Asp

185

180

ttg cta gag gct gga gcg cag gtt cgt gtg ctt ggg cca acg aca gac 624 Leu Leu Glu Ala Gly Ala Gln Val Arg Val Leu Gly Pro Thr Thr Asp 195 200 205

ccg gag aca gag acc gct tct cag ctc cag aca act gag ctt gcc acg
Pro Glu Thr Glu Thr Ala Ser Gln Leu Gln Thr Thr Glu Leu Ala Thr
210 220

ctg aca act gtg gat ccg
Leu Thr Thr Val Asp Pro
225 230

<210> 31

<211> 230

<212> PRT

<213> Toxoplasma gondii

<400> 31

Arg Arg Pro Tyr His Tyr Glu Met Leu Asp Ile Pro Ser Ile Arg Arg

1 5 10 15

Val Glu Leu Pro Gly Ala Gln Val Arg Met Pro Met Ala Lys Glu Leu 20 25 30

Val Arg Asp Trp Gly Ser Val Val Gln Gln Gln Thr Thr Ser Asp Ser 35 40 45

Ser Ser Asp Thr Pro Ala Thr Arg Ser Arg Ser Ala Glu Ala Leu Cys
50 55 60

Val Phe Ser Thr Pro Cys Thr Ala Asp Ser Asp Gln Arg Met Lys Gly 65 70 75 80

Arg His Tyr Pro Gln Ser Tyr His Thr Pro Arg Asp Ser Ala Thr Lys
85 90 95

Arg Glu Lys Pro Leu Lys Ser Thr Phe Ile Trp Gly Thr Thr Val Glu 100 105 110

Asp Arg Asn His Pro Ile Ser Pro Asp Pro Phe Ser Arg Leu Gln Gly
115 120 125

Cys Gly Gln Thr Leu Gln Asp Glu Leu Pro Ser Ala Arg Thr Arg Pro 130 135 140

Gly Trp Ala Ala Leu Asp Ser Arg Leu Lys Asn Lys Asp Pro Gln Ile

145 150 155 160

Ser Ala Gly Asp Glu Ala Ala Lys Val Asp Asp Thr Ser Ala Glu Pro 165 170 175

Cys Leu Gly Thr Val Pro Ser Phe Cys Arg Leu Val Thr Ser His Asp 180 185 190

Leu Leu Glu Ala Gly Ala Gln Val Arg Val Leu Gly Pro Thr Thr Asp 195 200 205

Pro Glu Thr Glu Thr Ala Ser Gln Leu Gln Thr Thr Glu Leu Ala Thr 210 215 220

Leu Thr Thr Val Asp Pro 225 230

<210> 32

<211> 313

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(162)

<400> 32

cgc agg aat aat cct gac ggt cag acg cag cgg ttc gtg cag aca gtg 48
Arg Arg Asn Asn Pro Asp Gly Gln Thr Gln Arg Phe Val Gln Thr Val
1 5 10 15

aag caa tgg cag agt gta aaa agc aga acc aga gcg tgt ctg tcg gcc 96
Lys Gln Trp Gln Ser Val Lys Ser Arg Thr Arg Ala Cys Leu Ser Ala
20 25 30

aaa gga aag aga agg caa atc aca cag cga ata aac ctc acc tct gtc 144 Lys Gly Lys Arg Arg Gln Ile Thr Gln Arg Ile Asn Leu Thr Ser Val 35 40 45

tcg cac ccc gaa gca acg taggagagcc actggtgccg ccactctgtg

Ser His Pro Glu Ala Thr

50

ctgacaaaaa agaaccggcc cttcttcggc aggggcgtag ccagtctgca gacatttcaa 252

tttcgaagcg accggaagca gtgaaatttc cagggaagac gcccaggaga cgtcaacagc 312

313

<210> 33

<211> 54

<212> PRT

<213> Toxoplasma gondii

<400> 33

Arg Arg Asn Asn Pro Asp Gly Gln Thr Gln Arg Phe Val Gln Thr Val

1 5 10 15

Lys Gln Trp Gln Ser Val Lys Ser Arg Thr Arg Ala Cys Leu Ser Ala
20 25 30

Lys Gly Lys Arg Arg Gln Ile Thr Gln Arg Ile Asn Leu Thr Ser Val $35 \hspace{1.5cm} 40 \hspace{1.5cm} 45$

Ser His Pro Glu Ala Thr 50

<210> 34

<211> 389

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(195)

<400> 34

cgc tct cac gga ggc gca agt gag ttt tgg ctt tac ctc ttg aga aaa 48
Arg Ser His Gly Gly Ala Ser Glu Phe Trp Leu Tyr Leu Leu Arg Lys
1 5 10 15

cgg aac tct cca gaa gat cct cgt tcc gtc cgt cct cca cgt ccg tgt 96
Arg Asn Ser Pro Glu Asp Pro Arg Ser Val Arg Pro Pro Arg Pro Cys
20 25 30

gtc ttt cga gag atg gac aaa cag aga agc aga atc aag aaa gga ttc 144 Val Phe Arg Glu Met Asp Lys Gln Arg Ser Arg Ile Lys Lys Gly Phe 35 40 45

gca ttt gca ctt ggg tct gtc ttt tac ttc caa ggt cgt gaa ttt cat 192 Ala Phe Ala Leu Gly Ser Val Phe Tyr Phe Gln Gly Arg Glu Phe His 50 55 60

gcg tgacgaataa gagagacagg agtaggccgc aacttctcgt ctcttggcag 245
Ala

65

tttccgattt ctcttccttc cgaagecett gctgccaage actccatccg gtccggttgg 305

totototoag gttottogag caatogacgo gatgttotot gotgtogatg ogggggottg 365

gcgtgtctgc atatctcttc cagg 389

<210> 35

<211> 65

<212> PRT

<213> Toxoplasma gondii

<400> 35

Arg Ser His Gly Gly Ala Ser Glu Phe Trp Leu Tyr Leu Leu Arg Lys

1 5 10 15

Arg Asn Ser Pro Glu Asp Pro Arg Ser Val Arg Pro Pro Arg Pro Cys
20 25 30

Val Phe Arg Glu Met Asp Lys Gln Arg Ser Arg Ile Lys Lys Gly Phe 35 40 45

Ala Phe Ala Leu Gly Ser Val Phe Tyr Phe Gln Gly Arg Glu Phe His
50 55 60

Ala 65

<210> 36

<211> 548

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(546)

<400> 36

cga tot tot tot cac cgt tog oto tto ttt oto too gtt gto tgc gtc 48
Arg Ser Ser His Arg Ser Leu Phe Phe Leu Ser Val Val Cys Val
1 5 10 15

ctc tcc cca ctg cct ctc gcc gtc cgc gtc gtt cgc ctc cgg ggq agc 96

I	,e11	Ser	D		_											, ,	-1/0398/
					Pro I				23					30			
			35	-, -	gag c Slu H	13 G	ry G	40	rne A	Mia A	irg i	Arg A	Ala A 45	la F	Pro	Arg	144
g A	cg t la P	tc c he L 50	tt c eu A	gg g rg G	ga co ly Ai	- 9 FI	eg a 10 T.	ca a hr S	gc c er L	tg c eu A	gt t rg S	ca t Ger S	cc c er G	ag a ln A	ga rg '	acg Thr	192
6	5					0	y AI	g Al	rg Se	er Pi	75	is M	et Ar	g C	ys E	Phe 80	240
				8	c ag r Se 5	r Are	а Су	s cy	's G1 9	u Ar O	g Ar	g Ly	's Ar	g Se 9	r A 5	la	288
			10	0	c caq u Glr	. GIL	ı se.	10	а Ly: 5	s Ly:	s Al	a Ar	g Pro	Se:	r As	sn	336
		115	5		ato : Ile	1112	120	ı sei	c Val	. Asp	Ar ₍	g Val	l Gln	Cys	5 G1	·y	384
caa Gln	Gln 130	gac Asp	tcg Ser	aaa Lys	agg Arg	tcg Ser 135	agg Arg	aga Arg	tgg Trp	ccg Pro	900 Ala 140	a Ala	tcg Ser	act Thr	tc Se	t r	432
145	-			*****	att Ile 150	Arg	GIY	Arg	Asn	Ser 155	Glu	Val	Pro	Arg	Va. 160	1 0	480
gac Asp	agg Arg	tcc Ser	gcc Ala	aag Lys 165	tcg Ser	cct Pro	acg Thr	cca Pro	ctc Leu 170	tcg Ser	aag Lys	aag Lys	ccg Pro	aaa Lys 175	ato Met	e :	528
cgg Arg	tct Ser	ctg Leu	ccg Pro 180	cat His	acg Thr	gc											548

<210> 37

<211> 182

<212> PRT

<213> Toxoplaşma gondii

<400> 37

Arg Ser Ser Ser His Arg Ser Leu Phe Phe Leu Ser Val Val Cys Val 1 5 10 15

Leu Ser Pro Leu Pro Leu Ala Val Arg Val Val Arg Leu Arg Gly Ser 20 25 30

Arg Gln Cys Gly Glu His Gly Gly Phe Ala Arg Arg Ala Ala Pro Arg 35 40 45

Ala Phe Leu Arg Gly Arg Pro Thr Ser Leu Arg Ser Ser Gln Arg Thr 50 55 60

Pro Arg Ser Ala Gln Met Arg Arg Arg Ser Pro His Met Arg Cys Phe 65 70 75 80

Cys Glu Thr Gly Ser Ser Ala Cys Cys Glu Arg Arg Lys Arg Ser Ala 85 90 95

Arg Asp Gly Asn Leu Gln Glu Ser Ala Lys Lys Ala Arg Pro Ser Asn 100 105 110

Pro Met Ser Lys Ala Ile His Ala Ser Val Asp Arg Val Gln Cys Gly
115 120 125

Gln Gln Asp Ser Lys Arg Ser Arg Arg Trp Pro Ala Ala Ser Thr Ser 130 135 140

Ala Gly Val Gln Ala Ile Arg Gly Arg Asn Ser Glu Val Pro Arg Val 145 150 155 160

Asp Arg Ser Ala Lys Ser Pro Thr Pro Leu Ser Lys Lys Pro Lys Met 165 170 175

Arg Ser Leu Pro His Thr 180

<?10> 38

<211> 310 ~

<212> DNA

<213> Toxoplasma gondii

<220>

<223> At locations 16, 24, 25, 31, 38, 45 and 57, Xaa = unknown

<220> <221> CDS <222> (1)..(285) <400> 38 cgg gat cca gct gca cct aac agc aca cag gct gtg gca gcc qct ygt Arg Asp Pro Ala Ala Pro Asn Ser Thr Gln Ala Val Ala Ala Ala Xaa 10 acc gtg gta gtg atg aaa acm gam gmw gaa gtg tcc ggt gac aac stc Thr Val Val Met Lys Xaa Xaa Glu Val Ser Gly Asp Asn Xaa 20 25 agt caa ccg ggt agg sgt ccg ccg tcg cca aag ccg caw acg acg aag Ser Gln Pro Gly Arg Xaa Pro Pro Ser Pro Lys Pro Xaa Thr Thr Lys 35 ttt ccg cgg aga gag tca cca gac srg cag ggg acg agg cgg aga act Phe Pro Arg Arg Glu Ser Pro Asp Xaa Gln Gly Thr Arg Arg Arg Thr 50 55 gaa age ega gge get gtt age agg gta tgg eea ggg gaa aac eag mga 240 Glu Ser Arg Gly Ala Val Ser Arg Val Trp Pro Gly Glu Asn Gln Xaar 65 75 aga ctg tct gcc gtc gac gat tcg ata ccg gct aac cca tcg ctt 285 Arg Leu Ser Ala Val Asp Asp Ser Ile Pro Ala Asn Pro Ser Leu 90 95 tgaacgggtg gcgccctgcg atccg 310 <210> 39 <211> 95 <212> PRT <213> Toxoplasma gondii <400> 39

Arg Asp Pro Ala Ala Pro Asn Ser Thr Gln Ala Val Ala Ala Xaa 10

Thr Val Val Met Lys Xaa Xaa Xaa Glu Val Ser Gly Asp Asn Xaa

Ser Gln Pro Gly Arg Xaa Pro Pro Ser Pro Lys Pro Xaa Thr Thr Lys 35 40

Phe Pro Arg Arg Glu Ser Pro Asp Xaa Gln Gly Thr Arg Arg Arg Thr

90

50

55

60

Glu Ser Arg Gly Ala Val Ser Arg Val Trp Pro Gly Glu Asn Gln Xaa 65 70 75 80

Arg Leu Ser Ala Val Asp Asp Ser Ile Pro Ala Asn Pro Ser Leu

<210> 40

<211> 220

<212> DNA

<213> Toxoplasma gondii

85

<220>

<221> CDS

<222> (1)..(219)

<400> 40

cgg gat cct tgc ctc agt gtc agg gac atc gag cgt atg ttc cgt ata 48
Arg Asp Pro Cys Leu Ser Val Arg Asp Ile Glu Arg Met Phe Arg Ile
1 5 10 15

tgt cac cat cgt tct ctg tct cgc ctc ctt ggc gcc tct gtt gct tgg 96
Cys His His Arg Ser Leu Ser Arg Leu Leu Gly Ala Ser Val Ala Trp
20 25 30

gat gca gtt gac tgc tct tcg gct tcg tcg cgc aca cac tgg tcc ttg 144
Asp Ala Val Asp Cys Ser Ser Ala Ser Ser Arg Thr His Trp Ser Leu
35 40 45

ctt gcg tct gag ctc cct tcc gaa cgg gtt ctt ttt cga ctg cag gtt 192 Leu Ala Ser Glu Leu Pro Ser Glu Arg Val Leu Phe Arg Leu Gln Val 50 55 60

ctt cta aaa ttg cca gtt ccc gat ccc g
Leu Leu Lys Leu Pro Val Pro Asp Pro
65 70

<210> 41

<211> 73

<212> PRT

<213> Toxoplasma gondii

<400> 41

Arg Asp Pro Cys Leu Ser Val Arg Asp Ile Glu Arg Met Phe Arg Ile
1 5 10 15

Cys His His Arg Ser Leu Ser Arg Leu Leu Gly Ala Ser Val Ala Trp 20 25 30

Asp Ala Val Asp Cys Ser Ser Ala Ser Ser Arg Thr His Trp Ser Leu 35 40 45

Leu Ala Ser Glu Leu Pro Ser Glu Arg Val Leu Phe Arg Leu Gln Val
50 55 60

Leu Leu Lys Leu Pro Val Pro Asp Pro 65 70

<210> 42

<211> 642

<212> DNA

<213> Toxoplasma gondii

<220>

<223> N= unknown at 19, 23, 27, 28, 29, 41, 86 and 88

<220>

 $\langle 223 \rangle$ Xaa = unknown at 7, 8, 9, 10, 14, 29 and 30

<220>

<221> CDS

<222> (1)..(102)

<400> 42

cgg cgg gaa acc atg gag ntt tna tan nnt aca act tcc ana tgc atg 48
Arg Arg Glu Thr Met Glu Xaa Xaa Xaa Xaa Thr Thr Ser Xaa Cys Met
1 5 10 15

gta ggt acc caa aat gca aac tat aga cac aaa caa ang naa aat acn 96 Val Gly Thr Gln Asn Ala Asn Tyr Arg His Lys Gln Xaa Xaa Asn Xaa 20 25 30

tgg ggg tgatgcanna nggggangtn ggggacagan aaatngtcct tcagttntca 152 Trp Gly

tctttgcccg cngcgtngan nacgcaatac agcgggcgca gcggctcatc acaccantac 212

acganttntg caaagaagca cntntcttct ctcttcangt ctctntacca cttctaccac 272

ctgcaccccc gcttcgtcca caaaacacat ttgaacgatg tgaccaaaat gatccacaa 332

aacacgattg tttcgtcaca tgaaacctca gcaaattcag gcgccaggac ggctccttca 392

aacgtctaat ccagagtcct ctccgctcaa aaacacgatt gtttcgtcac atggaacctc 452
agcaaattca ggcgccagga cggcctccct tcaaacgtcn taatccagag tcntttccgn 512
tccatcccca cnttntgccc nttcacgttt ccagtggtgg catgtcatcg tctcccctg 572
tcaacgtccc atcacctgag tacaggcgcg aagcagcgga cagctgttct tccatctccc 632
tgtattccgg

<210> 43

<211> 34

<212> PRT

<213> Toxoplasma gondii

<400> 43

Arg Arg Glu Thr Met Glu Xaa Xaa Xaa Xaa Thr Thr Ser Xaa Cys Met
1 5 10 15

Val Gly Thr Gln Asn Ala Asn Tyr Arg His Lys Gln Xaa Xaa Asn Xaa 20 25 30

Trp Gly

<210> 44

<211> 381

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(81)

<400> 44

cgg atc cac aaa aac acg att gtt tcg tca cat gga acc tca gca aat 48
Arg Ile His Lys Asn Thr Ile Val Ser Ser His Gly Thr Ser Ala Asn
1 5 10 15

tca ggc gcc agg acg gcc tcc ctt caa acg tcc taatccagag tcctctccgc 101 Ser Gly Ala Arg Thr Ala Ser Leu Gln Thr Ser 20 25

tecatececa cettetgece etteaegttt ceagtggtgg catgteateg tetececetg 161

<210> 45 <211> 27 <212> PRT <213> Toxoplasma gondii <400> 45

Arg Ile His Lys Asn Thr Ile Val Ser Ser His Gly Thr Ser Ala Asn 1 5 10 15

Ser Gly Ala Arg Thr Ala Ser Leu Gln Thr Ser 20 25

<210> 46
<211> 432
<212> DNA
<213> Toxoplasma gondii
<220>
<221> CDS
<222> (1)..(255)
<400> 46

ttt ttt tcg agg tgc cgg cgg gtg cca gag aac gtg aaa ctt ctg gtt 48
Phe Phe Ser Arg Cys Arg Arg Val Pro Glu Asn Val Lys Leu Leu Val
1 5 10 15

tac aag ttg att aac cct tct gtc gag gcc cgc ctg ctc gcc ttg caa 96
Tyr Lys Leu Ile Asn Pro Ser Val Glu Ala Arg Leu Leu Ala Leu Gln
20 25 30

gcg ata gag act ccg gaa tac agg gag atg gaa gaa cag ctg tcc gct 144
Ala Ile Glu Thr Pro Glu Tyr Arg Glu Met Glu Glu Gln Leu Ser Ala
35 40 45

get teg ege etg tae tea ggt gat ggg acg ttg aca ggg gga gae gat 192 Ala Ser Arg Leu Tyr Ser Gly Asp Gly Thr Leu Thr Gly Gly Asp Asp 50 55 60

gac atg cca cca ctg aaa cgt gaa ggg gca gaa ggt ggg gat gga gcg Asp Met Pro Pro Leu Lys Arg Glu Gly Ala Glu Gly Gly Asp Gly Ala 65 gag agg act ctg gat taggacgttt gaagggaggc cgtcctggcg cctqaatttg 295 Glu Arg Thr Leu Asp 85 ctgaggttcc atgtgacgaa acaatcgtgt ttttgagcgg agaggactct ggattaggac 355 gtttgaaggg aggccgtcct ggcgcctgaa tttgctgagg tttcatgtga cqaaacaatc 415 gtgtttttgt ggatccg 432 <210> 47 <211> 85 <212> PRT <213> Toxoplasma gondii <400> 47 Phe Phe Ser Arg Cys Arg Arg Val Pro Glu Asn Val Lys Leu Leu Val Tyr Lys Leu Ile Asn Pro Ser Val Glu Ala Arg Leu Leu Ala Leu Gln 25 Ala Ile Glu Thr Pro Glu Tyr Arg Glu Met Glu Glu Gln Leu Ser Ala 35 40 Ala Ser Arg Leu Tyr Ser Gly Asp Gly Thr Leu Thr Gly Gly Asp Asp 50 55 Asp Met Pro Pro Leu Lys Arg Glu Gly Ala Glu Gly Gly Asp Gly Ala 65 70 Glu Arg Thr Leu Asp 85 <210> 48 <211> 282 <212> DNA <213> Toxoplasma gondii <220> <221> CDS <222> (1)..(105)

| <400> 48 | |
|--|------|
| cgg cgg gct gct tcc cag gaa cgt ttc gcg gct gcg tgt gga cag caa | 48 |
| 1 grad Ala Sel Gin Glu Arg Phe Ala Ala Ala Cys Gly Gln Gln | 40 |
| 10 15 | |
| ago off acc off gag the for off get goo gao gto ggo gao gco | 96 |
| The Bed Gru Phe Ser Leu Val Ala Ala Asp Val Gly Asp Ala | 90 |
| 25 30 | |
| gcg aac tcc tgagatcaaa cacacaaaaa ggccctcgtt gaaacatccc | 145 |
| Ala Asn Ser | - 10 |
| | |
| cacgcacgag cagaaggacg cgagcaagaa aacgtotoca goottotott goggtogott 2 | 205 |
| | |
| gcaagcggga gtgtcgtctc cctctgtctt tctctgtgta ctcgaagccc agcgacttcc 2 | :65 |
| ttgtcgagtt tctccgg 2 | 82 |
| | |
| <210> 49 | |
| <211> 35
<212> PRT | |
| <213> Toxoplasma gondii | |
| | |
| <400> 49 | |
| Arg Arg Ala Ala Ser Gln Glu Arg Phe Ala Ala Ala Cys Gly Gln Gln 1 5 10 | |
| 13 | |
| Ser Leu Thr Leu Glu Phe Ser Leu Val Ala Ala Asp Val Gly Asp Ala | |
| 25 30 | |
| Ala Asn Ser | |
| 35 | |
| | |
| <210> 50
<211> 466 | |
| <212> DNA | |
| <213> Toxoplasma gondii | |
| <220> | |
| <223> Xaa = unknown at 3, 11 and 36 | |
| | |
| <220> <221> CDS | |
| <222> (1)(213) | |

| <400 |)> 50 | 0 | | | | | | | | | | | | | | |
|----------|-------|-----------|-------|----------|------------|-------------|-----------|-------|-------|-------|-------|------|-----------|-----------|------------|-----|
| | | | | | cgg | | | | | | | | | | | 48 |
| Pne
1 | Pne | хаа | Arg | Cys
5 | Arg | Arg | vaı | Pro | 10 | хаа | Val | Ьys | Phe | Trp
15 | Phe | |
| | | | | | | | | | | | | | | | | |
| | | | | | cct | | | | | | | | - | _ | | 96 |
| ASN | гÀг | Leu | 20 | ASN | Pro | Ser | vaı | 25 | Ala | Arg | Leu | Phe | A1a
30 | Leu | GIn | |
| | | | - | | | | | | | | | | | | | |
| | | | | | gaa | | | | | | | _ | _ | | - | 144 |
| MIA | TTe | 35 | хаа | Pro | Glu | ryr | Arg
40 | GIU | мет | GIU | GIU | 45 | Leu | Ser | Ala | |
| | | | | | | | | | | | | | | | _ | |
| | | | | | tca | | | | | | | | | | - | 192 |
| Ala | 50 | Arg | ьeu | Tyr | Ser | 55 | ASP | СТУ | | Leu | 60 | GIÀ | GIÀ | Asp | Asp | |
| | | | | | | | | | | | | | | | | |
| | | | | | gaa
Glu | | tgaa | gggg | gca ç | gaago | gtggg | g at | ggag | cgga | l | 243 |
| 45P | Mec | 110 | FIO | reu | 70 | 1111 | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| gagg | acto | tg g | jatta | iggac | g tt | tgaa | ggga | ggc | cgto | ctg | gcgc | ctga | at t | tgct | gaggt | 303 |
| tcca | tgtg | gac g | jaaac | aato | g tg | tttt | tgag | g cgc | gagag | gac | tctg | gatt | ag g | acgt | ttgaa | 363 |
| ~~~ | ~~~ | .~+ ~ | | | ·+ ~- | | aata | | | | +~~ | | | | | 422 |
| 3990 | ggcc | .gc c | ccgg | ,cycc | .c ga | accc | gerg | , ayu | , | acy | cyac | yaaa | ica a | recge | gtttt | 423 |
| tgtg | gato | ct c | ccga | ataca | aa aa | tcgg | ggta | cga | atac | agt | gtc | | | | | 466 |
| | | | | | | | | | | | | | | | | |
| <210 | > 51 | | | | | | | | | | | | | | | |
| | > 71 | | | | | | | | | | | | | • | | |
| | !> PF | T
xopl | asma | a aor | ndii | | | | | | | | | | | |
| | ,, 10 | ,,,opi | | . go. | | | | | | | | | | | | |
| | > 51 | | | _ | _ | | | _ | - 1 | | | _ | | _ | . . | |
| Phe
1 | Phe | Xaa | Arg | Cys
5 | Arg | Arg | Val | Pro | 10 | Хаа | Val | Lys | Phe | Trp | Phe | |
| | | | | | | | | | | | | | | | | |
| Asn | Lys | Leu | | Asn | Pro | Ser | Val | | Ala | Arg | Leu | Phe | | Leu | Gln | |
| | | | 20 | | | | | 25 | | | | | 30 | | | |
| Ala | Ile | | Xaa | Pro | Glu | Tyr | | Glu | Met | Glu | Glu | Gln | Leu | Ser | Ala | |
| | | 2 5 | | | | | A 0 | | | | | A [| | | | |

55

Ala Ser Arg Leu Tyr Ser Gly Asp Gly Thr Leu Thr Gly Gly Asp Asp

Asp Met Pro Pro Leu Glu Thr 65 70 <210> 52 <211> 539 <212> DNA <213> Toxoplasma gondii <220> <223> Xaa = unknown at 8, 9 and 16 <220> <221> CDS <222> (1)..(60) <400> 52 gat agc aca cgg aat gga tgc ntg grg gtt ggg agc gac tat att tnt 48 Asp Ser Thr Arg Asn Gly Cys Xaa Xaa Val Gly Ser Asp Tyr Ile Xaa tat ttg gtg ctt taaagctcca actacaggac ctgaagagga atactccatc 100 Tyr Leu Val Leu 20 gaattettgt teteattgtg eeggegggea eeagagaacg tgaaactact ggtttacaag 160 ttgattaacc cttctgtcga ggcccgcctg tcgccttgca agctacggag actccggaat 220 acagggagat ggaagaacag ctgtccgctg cttcgcgcct gtactcaggt gatgggacgt 280 tgacaggggg agacgatgac atgccaccac cggaaacgtg aaggggcaga aggtggggat 340 ggagcggaga ggactctgga ttaggacgtt tgaagggagg ccgtcctggc gcctgaattt 400 tgctgaggtt tcatgtgacg aaacaatcgt gtttttgtgg atccggaatt ccggatcggg 460 gaattteete teacaceget tggggeegag acacgegeag agaegttgtt gggeeteeae 520 aacacagggg ggattaagg 5.39

<210> 53 <211> 20 <212> PRT <213> Toxoplasma gondii

<400> 53

Asp Ser Thr Arg Asn Gly Cys Xaa Xaa Val Gly Ser Asp Tyr Ile Xaa l 5 10 15

Tyr Leu Val Leu 20

<210> 54

<211> 1233

<212> DNA

<213> Toxoplasma gondii

<400> 54

cgggatccct gaaggagagc atattectga agagtteeca gaaggegage atgtteetga 60 ggaggaaatc cctgaaggag aacatattcc tgaggaggag ttccctgaag gagagcatgt 120 teetgaggag gagateeetg aaggegagea tgtteetgag gaggagetee etqqaqqaqa 180 acttatteet gaggaggaga teeetgaagg agageatgtt eetgaagage teeetgaagg 240 cgagcatgtt cctgaggagg agatccctga aggagagcat gttcctgaag aggaaatccc 300 tgaaggcgag catgttcctg aggaggagat ccctgaagga gaacatgctc cagaggaaga 360 gactcctgca cctgaggaga ccgaaaagga ggaggaagaa ggcgtgccag tcgcagcgat 420 tgccggtggt gtcgtcggag gtgtgttgct cattgctggt ggtgcaggtg ctgccgtgta 480 cacaaaccaa ggtggcgttg aagcagctga agacgaagtg atgtttgaga gcgaagaaga 540 cggaacccag gctggcgaga accgcgagag gagacggtca ttgagatcga agatgacgca 600 tgggcagaca ttggactaaa ggagactagg aggtctgtgt gggcacatqc aggcqtqcqa 660 caaaaccgtg atcgcgaggt attctgtgtt acgggcggag cgtctgcggc tgtccttcga 720 aggggaggcg gagtgacact ctgagctagg taccagacga acgcagccat ttgtgtccqt 780 ccgctgtttc ttgatcctgg acacagacca gacccgacac gggtgctgaa cggtaatgca 840 aactgtcggg aaaacctctc cggcgcgaaa acagagattt acacaccgtt gagatctgag 900 tagcggaagt gcatgcgcat gtgtgtccac gagaaaggaa gattttcttt cgagaacgtt 960 tecettigti egeacatice taegegggge tegigeegea tgeatgiega gaggaetgeg 1020

ttcgagtgct ctttcgccgt tgcagtgct gacattgcgg cgtgggcaaa ggatagaagt 1080 gacgacetct gacatggcag tgaaggtggc agagactcgc ggaaaatcca aaaactctct 1140 gccgtttcgg tcgaggaatc acctttcttt ttttcgtctc tggacccgcc tccgtggtgt 1200 tcccttgccc ttgcaagccg ctgctatgta gcg 1233

<210> 55 <211> 411 <212> DNA <213> Toxoplasma gondii <220> <221> CDS <222> (1)..(180) <400> 55 cga cga cct cgg ctt ctc cac ata caa gga atg tct tcc tgt ttt gga Arg Arg Pro Arg Leu Leu His Ile Gln Gly Met Ser Ser Cys Phe Gly 1 10 15 cct aag caa ccc gac ctt tat ctt ttg cac cag ctg tgc ttc ttt tac 96 Pro Lys Gln Pro Asp Leu Tyr Leu Leu His Gln Leu Cys Phe Phe Tyr 20 ttg tgt gaa tca ctg tgt aaa caa act gag aag cgt gta tgc atg gtc 144 Leu Cys Glu Ser Leu Cys Lys Gln Thr Glu Lys Arg Val Cys Met Val 35 40 gcc ttt gca tgt gga cga ggc cgc cgt cgc aca gcg tgattctcat 190 Ala Phe Ala Cys Gly Arg Gly Arg Arg Arg Thr Ala 50 55 ctctgttgcg tgggggcgcg gatgagaatc aactccttag tgtcacagca tcagtgcagt 250 gcgtggagca acaattcttt tcgtgcacag acaagacaca ccagatatga aagaacacta 310 acgggcactt accgttgtcc gtctatatat ttatatttag tcaatgctga gattagacct 370 agacttgtga gagagagtgt gaaacccaaa tgcctagatc c 411

<210> 56 <211> 60 <212> PRT <213> Toxoplasma gondii

<400> 56 Arg Arg Pro Arg Leu Leu His Ile Gln Gly Met Ser Ser Cys Phe Gly 5 10 15 Pro Lys Gln Pro Asp Leu Tyr Leu Leu His Gln Leu Cys Phe Phe Tyr Leu Cys Glu Ser Leu Cys Lys Gln Thr Glu Lys Arg Val Cys Met Val Ala Phe Ala Cys Gly Arg Gly Arg Arg Arg Thr Ala 55 <210> 57 <211> 441 <212> DNA <213> Toxoplasma gondii <220> <223> Xaa = unknown at 51, 80 and 109 <220> <221> CDS <222> (1)..(354) <400> 57 egg ate gaa gaa get gaa geg gag aca ega ate gee gag aca ane uuu Arg Ile Glu Glu Ala Glu Ala Glu Thr Arg Ile Ala Glu Thr Gly Lys 1 5 10 cac age ggg aat gag aat ega ete tge gat aga agt ggg ege eut $q_{\mathrm{G}_{1}}$ His Ser Gly Asn Glu Asn Arg Leu Cys Asp Arg Ser Gly Arg His Giy 20 25 atc aag gaa cog agg oga agg occ atg otg ttg goo gag gtg occ Ile Lys Glu Pro Arg Arg Arg Pro Met Leu Leu Ala Glu Val Fro 35 40 tgc ttg tkg gag ggc gcc cga cga aca ggg ttt cgt cag aga cuu $q_{\omega^{\prime\prime}}$ 193 Cys Leu Xaa Glu Gly Ala Arg Arg Thr Gly Phe Arg Gln Arg Gln Ala 55 ctt ogc tog ogt ttg tgg occ ott geo gtg ogg oac geg tgc gta kec 240 Leu Arg Ser Arg Leu Trp Pro Leu Ala Val Arg His Ala Cys Val Xaa 65 70 75

ttc aag aga gac tgc gga agc aga gag agg cca ttg agg ctg tcc gag 288
Phe Lys Arg Asp Cys Gly Ser Arg Glu Arg Pro Leu Arg Leu Ser Glu
85 90 95

gtc ggc tcc agc cga gct gga tcc gaa tcc tgc agc csg gga tcc act 336 Val Gly Ser Ser Arg Ala Gly Ser Glu Ser Cys Ser Xaa Gly Ser Thr 100 105 110

agt cta gac gcg cac ccg tgacccactt caggaygcgg vmatwatrcm 384
Ser Leu Asp Ala His Pro
115

ggggcagatt tttwmggyta actatcattt ccccstwgtt gattmttcca gcaattg 441

<210> 58

<211> 118

<212> PRT

<213> Toxoplasma gondii

<400> 58

Arg Ile Glu Glu Ala Glu Ala Glu Thr Arg Ile Ala Glu Thr Gly Lys
1 5 10 15

His Ser Gly Asn Glu Asn Arg Leu Cys Asp Arg Ser Gly Arg His Gly 20 25 30

Ile Lys Glu Pro Arg Arg Arg Pro Met Leu Leu Ala Glu Val Pro
35 40 45

Cys Leu Xaa Glu Gly Ala Arg Arg Thr Gly Phe Arg Gln Arg Gln Ala 50 55 60

Leu Arg Ser Arg Leu Trp Pro Leu Ala Val Arg His Ala Cys Val Xaa 65 70 75 80

Phe Lys Arg Asp Cys Gly Ser Arg Glu Arg Pro Leu Arg Leu Ser Glu 85 90 95

Val Gly Ser Ser Arg Ala Gly Ser Glu Ser Cys Ser Xaa Gly Ser Thr 100 105 110

Ser Leu Asp Ala His Pro 115

<210> 59 <211> 491

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(102)

<400> 59

cgg cgg tat tat agg aca cgg ccg cct gct ggt aac atc tgt aat tta 48
Arg Arg Tyr Tyr Arg Thr Arg Pro Pro Ala Gly Asn Ile Cys Asn Leu
1 5 10 15

tca ttg tat ccc gtc gtc ccg tgt tcc aaa ctg gga atc ttt tct ttc 96
Ser Leu Tyr Pro Val Val Pro Cys Ser Lys Leu Gly Ile Phe Ser Phe
20 25 30

ctg agc tgacggtttg gcccgcaagc tcagccgagt acgaaaccat gattaggttg 152 Leu Ser

gaggectaat gtgetttte geeagetgte aaacgggeag ceaaggttga tttetetatg 212
agttgteete egegeteteg aattggtatt tegtggttte agattgaaag egteactega 272
getattacga ggegttteag caaaaaggaa gaateactea gacacetgae egaegettga 332
tgtgetggeg gttgtgeaaa teeaggeate aeteaacgee gatgeteage aggaeceatg 392
gatettaaga ggttetgtte eactacatea gtgagagttt caaaaagaat eetgataact 452
aegegettet aeaggtgeeg eetttatgge aacgateeg 491

<210> 60

<211> 34

<212> PRT

<213> Toxoplasma gondii

<400> 60

Arg Arg Tyr Tyr Arg Thr Arg Pro Pro Ala Gly Asn Ile Cys Asn Leu

1 5 10 15

Ser Leu Tyr Pro Val Val Pro Cys Ser Lys Leu Gly Ile Phe Ser Phe 20 25 30

Leu Ser

<210> 61 <211> 387 <212> DNA <213> Toxoplasma gondii <220> <221> CDS <222> (1)..(387) <400> 61 cgg atc gct ctg agt ctc ttt ggg ctc cct gcc gca tgc agg cat gaa Arg Ile Ala Leu Ser Leu Phe Gly Leu Pro Ala Ala Cys Arg His Glu agt gtc tcg ccg cga gag aca gag aag gaa gtg cag agc gag cgt ggg 96 Ser Val Ser Pro Arg Glu Thr Glu Lys Glu Val Gln Ser Glu Arg Gly 20 25 30 cga gaa cgg acg cag aaa ggc gca ggc gag aag gag acc ggc gta gac 144 Arg Glu Arg Thr Gln Lys Gly Ala Gly Glu Lys Glu Thr Gly Val Asp gga gtg act gga gag cag gtc tta gcg ctc act aag ggt gaa cct gaa 192 Gly Val Thr Gly Glu Gln Val Leu Ala Leu Thr Lys Gly Glu Pro Glu 50 55 240 Ala Ala Glu Glu Ala Arg Glu Glu Asp Glu Gly Lys Gly Glu Asp Arg 65 70 75 288 Trp Tyr Glu Glu Gly Ala Arg Arg Glu Lys Glu Ala Ala Arg Val Met 85 90 95 tcc act ccg cag acg tat gcc gaa gcc acc gac aca aca gct gca tgc 336 Ser Thr Pro Gln Thr Tyr Ala Glu Ala Thr Asp Thr Thr Ala Ala Cys 100 105 aga gac gaa agg gag ctc gcc tcg ggg gtc gaa gag aag aca cag gat 384 Arg Asp Glu Arg Glu Leu Ala Ser Gly Val Glu Glu Lys Thr Gln Asp 115 120 ccg 387 Pro

<210> 62 <211> 129

<212> PRT

<213> Toxoplasma gondii

<400> 62

Arg Ile Ala Leu Ser Leu Phe Gly Leu Pro Ala Ala Cys Arg His Glu
1 5 10 15

Ser Val Ser Pro Arg Glu Thr Glu Lys Glu Val Gln Ser Glu Arg Gly
20 25 30

Arg Glu Arg Thr Gln Lys Gly Ala Gly Glu Lys Glu Thr Gly Val Asp 35 40 45

Gly Val Thr Gly Glu Gln Val Leu Ala Leu Thr Lys Gly Glu Pro Glu 50 55 60

Ala Ala Glu Glu Ala Arg Glu Glu Asp Glu Gly Lys Gly Glu Asp Arg
65 70 75 80

Trp Tyr Glu Glu Gly Ala Arg Arg Glu Lys Glu Ala Ala Arg Val Met 85 90 95

Ser Thr Pro Gln Thr Tyr Ala Glu Ala Thr Asp Thr Thr Ala Ala Cys 100 105 110

Arg Asp Glu Arg Glu Leu Ala Ser Gly Val Glu Glu Lys Thr Gln Asp 115 120 125

Pro

<210> 63

<211> 417

<212> DNA

<213> Toxoplasma gondii

<220>

<223> N = unknown at 72, 74, 139 and 141

<220>

<223> At locations 25 and 47, Xaa = unknown

<220>

<221> CDS

<222> (1)..(417)

<223> Xaa = unknown at 25 and 47

<400> 63 ctt gca tgc gct gtg gca atg gaa gaa gca ccc gcg cca ggg caa cca 48 Leu Ala Cys Ala Val Ala Met Glu Glu Ala Pro Ala Pro Gly Gln Pro 1 15 ccc gaa gaa ggg gac gat ggc ggn tnt cag cag cgc ctg gag atc gct 96 Pro Glu Glu Gly Asp Asp Gly Xaa Xaa Gln Gln Arg Leu Glu Ile Ala 25 ctg agt ctc ttt ggg ctc cct gcc gca tgc agg cat gaa agt ntn tcq Leu Ser Leu Phe Gly Leu Pro Ala Ala Cys Arg His Glu Ser Xaa Ser 35 40 ccg cga gag aca gag aag gaa gtg cag agc gag cgt ggg cga gaa cgg 192 Pro Arg Glu Thr Glu Lys Glu Val Gln Ser Glu Arg Gly Arg Glu Arg 50 55 acg cag aaa ggc gca ggc gag aag gag acc ggc gta gac gga gtg act 240 Thr Gln Lys Gly Ala Gly Glu Lys Glu Thr Gly Val Asp Gly Val Thr 65 70 75 gga gag cag ctc tta gcg ctc act aag ggt gaa cct gaa gcg gca gaa Gly Glu Gln Leu Leu Ala Leu Thr Lys Gly Glu Pro Glu Ala Ala Glu 85 90 Glu Ala Arg Glu Glu Asp Glu Gly Lys Gly Glu Asp Arg Trp Asn Glu 100 105 110 gaa ggc gcg agg cga gag aaa gag gcg gct cga gtc atg tcc act ccq 384 Glu Gly Ala Arg Arg Glu Lys Glu Ala Ala Arg Val Met Ser Thr Pro 115 120 125 cag acg tat gcc gaa gcc acc gac aca aca gcg 417 Gln Thr Tyr Ala Glu Ala Thr Asp Thr Thr Ala 130 135 <210> 64 <211> 139 <212> PRT <213> Toxoplasma gondii <400> 64

Leu Ala Cys Ala Val Ala Met Glu Glu Ala Pro Ala Pro Gly Gln Pro 10

Pro Glu Glu Gly Asp Asp Gly Xaa Xaa Gln Gln Arg Leu Glu Ile Ala

20 25 30

Leu Ser Leu Phe Gly Leu Pro Ala Ala Cys Arg His Glu Ser Xaa Ser 35 40 45

Pro Arg Glu Thr Glu Lys Glu Val Gln Ser Glu Arg Gly Arg Glu Arg 50 55 60

Thr Gln Lys Gly Ala Gly Glu Lys Glu Thr Gly Val Asp Gly Val Thr 65 70 75 80

Gly Glu Gln Leu Leu Ala Leu Thr Lys Gly Glu Pro Glu Ala Ala Glu 85 90 95

Glu Ala Arg Glu Glu Asp Glu Gly Lys Gly Glu Asp Arg Trp Asn Glu 100 105 110

Glu Gly Ala Arg Arg Glu Lys Glu Ala Ala Arg Val Met Ser Thr Pro 115 120 125

Gln Thr Tyr Ala Glu Ala Thr Asp Thr Thr Ala 130 135

<210> 65

<211> 416

<212> DNA

<213> Toxoplasma gondii

<220>

<223> N = unknown at 74 and 107

<220>

<221> CDS

<222> (1)..(414)

<400> 65

ccg gat cgc ggg aga gaa gaa cgt gag gga gaa gaa gag agt gcc gag 48
Pro Asp Arg Gly Arg Glu Glu Arg Glu Gly Glu Glu Glu Ser Ala Glu
1 5 10 15

gct ttg cca gac cat aag cgg ggg cca gga aaa gag ctg gag gaa ggc 96
Ala Leu Pro Asp His Lys Arg Gly Pro Gly Lys Glu Leu Glu Glu Gly
20 25 30

cga gac tcg cag gtc cgt ggt gag gag agc ggg cgc agc tcg ctt tcg 144
Arg Asp Ser Gln Val Arg Gly Glu Glu Ser Gly Arg Ser Ser Leu Ser
35 40 45

| | gag
Glu
50 | | | | | | | | | | | | | | | 192 |
|------------|----------------------------------|------------|------------|------------------|------------|------------|------------|------------|------------------|------------|------------|------------|------------|------------------|------------|-----|
| | gtg
Val | | | | | | | | | | | | | | | 240 |
| gac
Asp | aga
Arg | ccc
Pro | gac
Asp | ggc
Gly
85 | gag
Glu | ayc
Ser | aac
Asn | gag
Glu | ctg
Leu
90 | cgt
Arg | cgc
Arg | ttg
Leu | tca
Ser | ccc
Pro
95 | acc
Thr | 288 |
| | cag
Gln | | | | | | | | | | | | | | | 336 |
| | acg
Thr | | | | | | | | | | | | | | | 384 |
| | gag
Glu
130 | | | | | | | | | tt | | | | | | 416 |
| <21: | 0> 66
1> 13
2> PF
3> To | 38
RT | lasm | a gor | ndii | | | | | | | | | | | |
| | 0> 66
Asp | | Gly | Arg
5 | Glu | Glu | Arg | Glu | Gly
10 | Glu | Glu | Glu | Ser | Ala
15 | Glu | |
| Ala | Leu | Pro | Asp
20 | His | Lys | Arg | | Pro
25 | | Lys | Glu | Leu | Glu
30 | Glu | Gly | |
| Arg | Asp | Ser
35 | | Val | Arg | Gly | Glu
40 | Glu | Ser | Gly | Arg | Ser
45 | Ser | Leu | Ser | |
| Gln | Glu
50 | Arg | Glu | Ser | Phe | Arg
55 | Ser | Gln | Xaa | Val | Ser
60 | | Glu | Gly | Gln | |
| Glu
65 | Val | Glu | Ala | ı Xaa | Ser
70 | | Lys | Ala | Leu | Glu
75 | | Ala | Lys | Ser | Asn
80 | |
| Asr | Arq | Pro | Asp | Gly | Glu | Ser | Asn | Glu | ı Leu | Arg | Arg | Leu | Ser | Pro | Thr | |

90 95

Ser Gln Thr Glu Gln Glu Gly Ser Val Glu Lys Glu Gly Thr Ser Glu 100 105 110

Ala Thr Met Asn Asp Gln Asp Glu Thr Gly Lys Glu Lys Gln Asp Gln
115 120 125

Arg Glu Val Pro Val Pro Arg Ala Leu Arg 130 135

<210> 67

<211> 500

<212> DNA

<213> Toxoplasma gondii

<400> 67

ccgagaatca tgttacgcca tgtagacagc gtttagggag tgcagacatt ttaatctgga 60 cggagtccaa gtggacgcgg atgtagatat ctgtcgcagc acctccgcag ttgcgctagg 120 gattctgatg ctgctagttt taacatccaa aactctgact tcgcttggtg atctccaggt 180 gcatatacat gcgaaggcaa tcgtgttgt gagaggcgaa tgtacgaatt tcagtgtctt 240 tgtgtggaag tcaagttccc ctgaaccagc tgcttgttt attctaccgc taatgtatga 300 agcttagcct cgtgtcctct tcgcccgtac acgagacacg atccaagagt catacaaatt 360 cttgcggcgg tgaggtaatt gtcaacagaa acaaaagtcg cgggtatctg tggtgtctct 420 gcttctgcac ttccaaggac cgccgcaagt tcggcccgat cggctggaac attcagtacg 480 agttcacgac ggaggatccg

<210> 68

<211> 321

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(219)

<400> 68

egg egg gae ttg egg act teg gte tgg gae get egg gtg tae gta eac 48

| Arg
1 | Arg | Asp | Leu | Arg
5 | Thr | Ser | Val | Trp | Asp
10 | Ala | Arg | Val | Tyr | Val
15 | His | |
|------------|----------------------------------|-----------|-----------|----------|------------------|-----------|-----------|-----------|-----------|-------|-----------|------------------|-----------|-----------|--------|-----|
| | | | | | | | | | | | | ggg
Gly | | | | 96 |
| | | | | | | | | | | | | tgg
Trp
45 | | | | 144 |
| | | | | | | | | | | | | aca
Thr | | | | 192 |
| - | | | | | aaa
Lys
70 | | | | tgaa | iggaa | aat o | cacaç | gacat | c | | 239 |
| acca | acct | tc o | cgc | cgtgo | gc ta | aagg | jacco | , tcc | etgtg | gtat | gtac | agtt | tt t | ccaç | ggcgaa | 299 |
| agco | gaga | iga (| cage | gaaac | c go | Į | | | | | | | | | • | 321 |
| <21
<21 | 0> 69
L> 73
2> PE
3> To | 3
RT | lasma | a goi | ndii | | | | | | | | | | | |
| | 0> 69
Arg | | Leu | Arg
5 | Thr | Ser | Val | Trp | Asp
10 | Ala | Arg | Val | Tyr | Val
15 | His | |
| Leu | Ala | Gly | Gly
20 | | Arg | Arg | Cys | Asn
25 | Glu | Ser | Arg | Gly | Met
30 | | Glu | |
| Ala | Arg | Lys
35 | | Arg | Cys | Leu | Ala
40 | | Arg | Cys | Gln | Trp
45 | | Ser | Ser | |
| Ala | Leu
50 | | Trp | Arg | Glu | Ser
55 | | Lys | Asn | Ala | Glu
60 | | Ala | Ser | His | |
| Val | | Phe | Pro | Thr | Lys
70 | | Pro | Pro | | | | | | | | |
| | .0> 7
.1> 5 | | | | | | | | | | | | | | | |

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(513)

<400> 70

cgg gat cag gct tct atg cca ctg ccc ccg gcc ccc gaa gac ttt gac 48
Arg Asp Gln Ala Ser Met Pro Leu Pro Pro Ala Pro Glu Asp Phe Asp
1 10 15

ctg cct cct atg cca ctg ccc gaa gca ccc gaa gac ttt gac cag gct 96
Leu Pro Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala
20 25 30

cct atg cca ctg ccc gag gca ccc gaa gac ttt gac cag gct cct atg 144
Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala Pro Met
35 40 45

cca ctg ccc gag gca ccc gaa gac ttt gac cag cct cct atg cca ctg 192
Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Pro Pro Met Pro Leu
50 55 60

ccc gaa gca ccc gaa gac ttt gac cag gct cct atg cca ctg ccc gaa 240
Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala Pro Met Pro Leu Pro Glu
65 70 75 80

gca ccc gaa gtc ttt gac cag gct cct atg cca ctg ccc gag gca ccc 288
Ala Pro Glu Val Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro
85 90 95

gaa gtc ttt gac cag gct cct atg cca ctg ccc gaa gca ccc gaa gac 336 Glu Val Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Asp 100 105 110

ttt gac cag gct cct atg cca ctg ccc gaa gca ccc gaa gtc ttt gac 384
Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Val Phe Asp
115 120 125

cág gct cct atg cca ctg ccc gag gca ccc gaa gac ttt gac cag gct 432 Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala 130 135 140

cct atg cca gtg ccc gag gca ccc gaa gac ttt gac cag gct cct gag 480 Pro Met Pro Val Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala Pro Glu 145 150 155 160

cca ctg ccc gag gca gcc gaa gaa ttt gat ccc Pro Leu Pro Glu Ala Ala Glu Glu Phe Asp Pro 165 170 513

<210> 71

<211> 171

<212> PRT

<213> Toxoplasma gondii

<400> 71

Arg Asp Gln Ala Ser Met Pro Leu Pro Pro Ala Pro Glu Asp Phe Asp 1 5 10 15

Leu Pro Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala 20 25 30

Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala Pro Met
35 40 45

Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Pro Pro Met Pro Leu 50 55 60

Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala Pro Met Pro Leu Pro Glu 65 70 75 80

Ala Pro Glu Val Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro 85 90 95

Glu Val Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Asp 100 105 110

Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Val Phe Asp 115 120 125

Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala 130 135 140

Pro Met Pro Val Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala Pro Glu 145 150 155 160

Pro Leu Pro Glu Ala Ala Glu Glu Phe Asp Pro 165 170

<210> 72

<211> 528

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(528)

<400> 72

cga tct gaa cgt tgt gca acc gtt ggg gac cca ggt aca ggc gtc tcc 48
Arg Ser Glu Arg Cys Ala Thr Val Gly Asp Pro Gly Thr Gly Val Ser
1 5 10 15

aac act gag gcg ggg gga aag cgc cca cac tgg cgt ctc agg cac ctt 96
Asn Thr Glu Ala Gly Gly Lys Arg Pro His Trp Arg Leu Arg His Leu
20 25 30

caa tgc cac agg tat ccg gca tcc ttg gag aca gag ctt gag acg qug 144 Gln Cys His Arg Tyr Pro Ala Ser Leu Glu Thr Glu Leu Glu Thr Glu 35 40 45

aca ctc gca cac aca ccc aga gag ctt gtg gtg aca aat cga auc ttn — 192 Thr Leu Ala His Thr Pro Arg Glu Leu Val Val Thr Asn Arg Ser Leu 50 — 55 — 60

ggg ttt gtc tcg ctt ctt cgc cag tcg ttc gcg tcg cag tca aaa aaa 240 Gly Phe Val Ser Leu Leu Arg Gln Ser Phe Ala Ser Gln Ser Gia Ala 65 70 75 80

gtc aag gcg acc gcg gag acg ccg aca gag aca gag aca gtc ctt gt; 288
Val Lys Ala Thr Ala Glu Thr Pro Thr Glu Thr Glu Thr Val Leu Val
85 90 95

gcg ggc gag cgc aac acc gcg aaa gaa aga gag aga aaa ggg can guc 336 Ala Gly Glu Arg Asn Thr Ala Lys Glu Arg Glu Arg Lys Gly Glr Arg 100 105 110

gaa gag gtt tcg cag aga gca gcg gag aac aag aga gga cga gtg ga: 584 Glu Glu Val Ser Gln Arg Ala Ala Glu Asn Lys Arg Gly Arg Val 31. 115 120 125

gac aca gac tac cgg gag acg gat aag aaa gcc gag aaa gat dig c:... 432 Asp Thr Asp Tyr Arg Glu Thr Asp Lys Lys Ala Glu Lys Asp Glu Ar; 130 135 140

gaa gag aac ccc cga gga gac aca ggg gag cag aga agc gag aan cuc 480 Glu Glu Asn Pro Arg Gly Asp Thr Gly Glu Gln Arg Ser Glu Lys His 145 150 150

acg aga gat tta ttg gga cag gag aga gag aac gca tgg gag atc ccg 52%

Thr Arg Asp Leu Leu Gly Gln Glu Arg Glu Asn Ala Trp Glu Ile Pro 165 170 175

<210> 73

<211> 176

<212> PRT

<213> Toxoplasma gondii

<400> 73

Arg Ser Glu Arg Cys Ala Thr Val Gly Asp Pro Gly Thr Gly Val Ser 1 5 10 15

Asn Thr Glu Ala Gly Gly Lys Arg Pro His Trp Arg Leu Arg His Leu 20 25 30

Gln Cys His Arg Tyr Pro Ala Ser Leu Glu Thr Glu Leu Glu Thr Glu 35 40 45

Thr Leu Ala His Thr Pro Arg Glu Leu Val Val Thr Asn Arg Ser Leu 50 55 60

Gly Phe Val Ser Leu Leu Arg Gln Ser Phe Ala Ser Gln Ser Glu Ala 65 70 75 80

Val Lys Ala Thr Ala Glu Thr Pro Thr Glu Thr Glu Thr Val Leu Val 85 90 95

Ala Gly Glu Arg Asn Thr Ala Lys Glu Arg Glu Arg Lys Gly Gln Asp 100 105 110

Glu Glu Val Ser Gln Arg Ala Ala Glu Asn Lys Arg Gly Arg Val Glu 115 120 125

Asp Thr Asp Tyr Arg Glu Thr Asp Lys Lys Ala Glu Lys Asp Glu Arg 130 135 140

Glu Glu Asn Pro Arg Gly Asp Thr Gly Glu Gln Arg Ser Glu Lys His 145 150 155 160

Thr Arg Asp Leu Leu Gly Gln Glu Arg Glu Asn Ala Trp Glu Ile Pro 165 170 175

<210> 74

<211> 375

<212> DNA

<213> Toxoplasma gondii

<220> <221> CDS <222> (1)..(375) <400> 74 ccg gag gag tac aag tgc agc aaa acc acg tac gaa gac agc tgc acc Pro Glu Glu Tyr Lys Cys Ser Lys Thr Thr Tyr Glu Asp Ser Cys Thr 10 gat gtc gct gtc cag gtc ccc gac acc tgc tac cgc act gtc gat cag Asp Val Ala Val Gln Val Pro Asp Thr Cys Tyr Arg Thr Val Asp Gln 20 aag aag get tac aag tge aag aaa acg etg acg aaa aac caa tge acg Lys Lys Ala Tyr Lys Cys Lys Lys Thr Leu Thr Lys Asn Gln Cys Thr 40 aag gtt cca gtc cag gtt cca agc aca tgc acg aag acg gcg atg tca 192 Lys Val Pro Val Gln Val Pro Ser Thr Cys Thr Lys Thr Ala Met Ser 50 55 aag gag geg tae gae tge teg aag ace gag tte ege ace gag tge ace 240 Lys Glu Ala Tyr Asp Cys Ser Lys Thr Glu Phe Arg Thr Glu Cys Thr 70 75 gac gaa gtc gag caa gtc ccg tgc atg ggc aaa gag tgc aag ctg cqc Asp Glu Val Glu Gln Val Pro Cys Met Gly Lys Glu Cys Lys Leu Arg 85 90 cag ctg aag aag aag cgc gtc tgc agg cag gtc ccg ttc acc agc aag 336 Gln Leu Lys Lys Lys Arg Val Cys Arg Gln Val Pro Phe Thr Ser Lys 100 105 aac gtc tgc tac aaa aat gtg ccc acg gag cag acg tcg 375. Asn Val Cys Tyr Lys Asn Val Pro Thr Glu Gln Thr Ser 115 120 125

<210> 75

<211> 125

<212> PRT

<213> Toxoplasma gondii

<400> 75

Pro Glu Glu Tyr Lys Cys Ser Lys Thr Thr Tyr Glu Asp Ser Cys Thr 1 5 10 15

Asp Val Ala Val Gln Val Pro Asp Thr Cys Tyr Arg Thr Val Asp Gln
20 25 30

Lys Lys Ala Tyr Lys Cys Lys Lys Thr Leu Thr Lys Asn Gln Cys Thr 35 40 45

Lys Val Pro Val Gln Val Pro Ser Thr Cys Thr Lys Thr Ala Met Ser 50 55 60

Lys Glu Ala Tyr Asp Cys Sei Lys Thr Glu Phe Arg Thr Glu Cys Thr 65 70 75 80

Asp Glu Val Glu Gln Val Pro Cys Met Gly Lys Glu Cys Lys Leu Arg 85 90 95

Gln Leu Lys Lys Lys Arg Val Cys Arg Gln Val Pro Phe Thr Ser Lyc 100 105 110

Asn Val Cys Tyr Lys Asn Val Pro Thr Glu Gln Thr Ser 115 120 125

<210> 76

<211> 543

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(267)

<400> 76

cga too aac agt tta cga ggt aca agg caa cag ccg aac ctc tac quq. 48
Arg Ser Asn Ser Leu Arg Gly Thr Arg Gln Gln Pro Asn Leu Tyr Glu
1 5 10 15

cac gtg tcc cca cgg ttc acg ctc tcc cat gga aaa gca aag cga tt: 96 His Val Ser Pro Arg Phe Thr Leu Ser His Gly Lys Ala Lys Arg Phe 20 25 30

ctc cat tat cac cac tgc cac tgc cat tcc age cta aga atc cta cac 144 Leu His Tyr His His Cys His Cys His Ser Ser Leu Arg Ile Leu His 35 40 45

ttc aaa gac gaa ctt ttg cat cgt ccg tgc gtc tcc cgt ggc caa cac 192
Phe Lys Asp Glu Leu Leu His Arg Pro Cys Val Ser Arg Gly Gln His
50 55 60

cct caa gcc aaa aga gag ggc acc ttc tac act gcc cac gca atc acc 240 Pro Gln Ala Lys Arg Glu Gly Thr Phe Tyr Thr Ala His Ala Ile Thr 65 70 75 80

ctg tgc ggc ggc aca caa aag cga aac tgacacacgc tactgccgtt 287 Leu Cys Gly Gly Thr Gln Lys Arg Asn
85

ccggaaagtg gtctgaaaga aactgacaac agccgcaaag agacatttac ccggtgcctg 347
gcgtggtcaa aaatccggca taatggttte tgcgcatcct ccattcagce gcccaacate 407
tgcggtcgtt cttccgtcga aactatgaca caacgagcct tgtggaacaa aacggttcgt 467
actgacgaca ttgcctgggt cggattcact gcatgtttge cagggtgcat ttccacggtg 527
ctctgcgtcg atcccg

<210> 77

<211> 89

<212> PRT

<213> Toxoplasma gondii

<400> 77

Arg Ser Asn Ser Leu Arg Gly Thr Arg Gln Gln Pro Asn Leu Tyr Glu
1 5 10 15

His Val Ser Pro Arg Phe Thr Leu Ser His Gly Lys Ala Lys Arg Phe 20 25 30

Leu His Tyr His His Cys His Cys His Ser Ser Leu Arg Ile Leu His $35 \hspace{1cm} 40 \hspace{1cm} 45$

Phe Lys Asp Glu Leu Leu His Arg Pro Cys Val Ser Arg Gly Gln His
50 55 60

Pro Gln Ala Lys Arg Glu Gly Thr Phe Tyr Thr Ala His Ala Ile Thr 65 70 75 80

Leu Cys Gly Gly Thr Gln Lys Arc Asn 85

<210> 78

<211> 573

<212> DNA

<213> Toxoplasma gondii

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165 170 175

ccg ttg cag gac gat tct gaa gga gtc caa caa cct ctt ccg ccg 573
Pro Leu Gln Asp Asp Ser Glu Gly Val Gln Gln Pro Leu Pro Pro
180 185 190

<210> 79

<211> 191

<212> PRT

<213> Toxoplasma gondii

<400> 79

Pro Ala Ser Ser Ser Ser Arg Leu Gly Lys Leu Ala Tyr Asp Asp Ala 1 5 10 15

Gly Gly Gly Arg Gly Ala Ser Ser Pro Pro Ser Ser Lys Leu Phe Val 20 25 30

Ser Pro Val Asn Asp Arg Ser Arg Met Ala Asp Gln Arg Lys Pro Ala 35 40 45

Pro Glu Gln Ser Ser Asn His Asp Ser Glu Cys Cys Cys Leu Arg Cys
50 55 60

Leu Ser Glu Lys Thr Leu Met Met Ala Gln Leu Cys Arg Pro Ala Pro 65 70 75 80

Val Thr Leu Ser Val Thr Glu Arg Asn Leu Phe Gly Asp Asn Gly Arg 85 90 95

Asp Val Val Glu Trp Glu Gly Ser Cys Gly Phe Phe Ser Gly Asn Ala 100 105 110

Ser Thr Arg Pro Ser Leu Gln Phe Ser Pro His Arg Val Ile Asp Ala 115 120 125

Pro Thr Ala Asn Asp Asp Met Arg Asp Cys Arg Ala Ala Pro Glu Asp 130 135 140

Gly Thr Gly Thr Ser Lys Ala Asn Ile His Arg Ser Ser Asn Ile Thr 145 150 155 160

Lys Thr Lys Glu Glu Asn Gly Arg Asp Val Cys Glu Gly Leu Arg Lys 165 170 175

Pro Leu Gln Asp Asp Ser Glu Gly Val Gln Gln Pro Leu Pro Pro 180 185 190

| <210 | | | | | | | | | | | | | | | | |
|------|------|------|------|-----|-----|-----|-------|-------|-----|-----|-----|-----|-----|-----|-----|-----|
| <211 | | | | | | | | | | | | | | | | |
| <212 | | | | | | | | | | | | | | | | |
| <213 | > To | xopl | asma | gon | dii | | | | | | | | | | | |
| <220 | > | | | | | | | | | | | | | | | |
| <221 | > CD | S | | | | | | | | | | | | | | |
| <222 | > (1 |) (| 1833 |) | | | | | | | | | | | | |
| <400 | | | | | | | | | | | | | | | | |
| cgg | atc | agt | ggg | gac | cag | tac | tct | tgt | ctt | caa | cga | gga | gcg | gga | gga | 48 |
| Arg | Ile | Ser | Gly | Asp | Gln | Tyr | Ser | Суѕ | | Gln | Arg | Gly | Ala | | GIA | |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | | |
| gac | aag | gag | aca | gca | acc | gag | aga | gaa | gag | agg | aac | aga | gaa | gat | gcg | 96 |
| Asp | Lys | Glu | Thr | Ala | Thr | Glu | Arg | Glu | Glu | Arg | Asn | Arg | | Asp | Ala | |
| | | | 20 | | | | | 25 | | | | | 30 | | | |
| ccc | tcc | ttt | ctt | gaa | gga | gga | ctc | gga | gat | gac | gag | aca | gag | aga | gcg | 144 |
| Pro | Ser | Phe | Leu | Glu | Gly | Gly | Leu | Gly | Asp | Asp | Glu | Thr | Glu | Arg | Ala | |
| | | 35 | | | | | 40 | | | | | 45 | | | • | |
| aag | caa | gcg | agt | gag | ttg | ccc | gcg | tct | ctt | tgc | tct | ttc | gcc | gca | gca | 192 |
| Lys | Gln | Ala | Ser | Glu | Leu | Pro | Ala | Ser | Leu | Cys | Ser | Phe | Ala | Ala | Ala | |
| | 50 | | | | | 55 | | | | | 60 | | | | | |
| cac | agg | aac | gcg | agc | cgc | gca | gag | aag | aca | ggc | gca | aag | ggg | gag | gaa | 240 |
| Ara | Ara | Glv | Ala | Ser | Arg | Ala | Glu | Lys | Thr | Gly | Ala | Lys | Gly | Glu | Glu | |
| 65 | | • | | | 70 | | | | | 75 | | | | | 80 | |
| gcc | aga | gag | aaa | gaa | gtc | agt | ttc | ggt | gaa | gac | agt | ggg | cta | tcc | aga | 288 |
| Ala | Arg | Glu | Lys | Glu | Val | Ser | Phe | Gly | | Asp | Ser | Gly | Leu | Ser | Arg | |
| | | | | 85 | | | | | 90 | | | | | 95 | | |
| cag | gtg | gac | atg | gac | agt | tcg | cag | gaa | tct | gtc | aac | gaa | gga | gag | ccg | 336 |
| Gln | Val | Asp | Met | Asp | Ser | Ser | Gln | Glu | Ser | Val | Asn | Glu | Gly | Glu | Pro | |
| | | | 100 | | | | | 105 | | | | | 110 | | | |
| cta | cac | gac | aga | gcc | gca | ggg | gag | gac | gca | gaa | ggc | ggg | gga | gca | gag | 384 |
| Leu | His | Asp | Arg | Ala | Ala | Gly | Glu | Asp | Ala | Glu | Gly | Gly | Gly | Ala | Glu | |
| | | 115 | | | | | 120 | | | | | 125 | | | | |
| aca | aac | gac | gga | gac | aga | gag | ı gga | gac | gag | aag | gag | act | cga | gac | gtc | 432 |
| Ala | Asn | Asp | Gly | Asp | Arg | Glu | G1, | / Asp | Glu | Lys | Glu | Thr | Arg | Asp | Val | |
| | 130 | | - | | | 135 | | | | | 140 | | | | | |

| | | | | | acg
Thr
150 | | | | | | | _ | | | 480 |
|---|---|---|---|---|-------------------|---|---|---|---|-------|---|---|---|-----|------|
| | | | | | gag
Glu | | | | | | | | _ | | 528 |
| | | | | | aat
Asn | | | | | | | | | | 576 |
| | | | | | tgt
Cys | | | | | | | _ | | - | 624 |
| | | | | | tct
Ser | | | | | | | | | - | 672 |
| | | | | | gat
Asp
230 | | | | | | | | | | 720 |
| | | | | | ggc
Gly | | | | | _ | - | | - | | 768 |
| _ | - | _ | _ | | ggt
Gly | | - | - | | ٠. | - | | | - | 816 |
| | - | | | | gaa
Glu | _ | | _ | |
- | - | - | _ | | 864 |
| | | | | | gaa
Glu | | | | | | | - | | _ | 912 |
| | - | _ | _ | _ | gaa
Glu
310 | | - | - | _ |
_ | | | - | , , | 960 |
| | | | | | cag
Gln | | | | | | | | - | | 1008 |

| gag
Glu | | | | | | | | | | | 1056 |
|-------------------|---|-------------------|-----|-----|--|-----|-----|--|--|--|------|
| aga
Arg | - | | - | - | | | | | | | 1104 |
| cgc
Arg | - | | | | | | | | | | 1152 |
| gag
Glu
385 | | | | | | | | | | | 1200 |
| gaa
Glu | | | | | | | | | | | 1248 |
| cag
Gln | - | | | | | | | | | | 1296 |
| | | aga
Arg
435 | | | | | | | | | 1344 |
| | | gag
Glu | | | | | | | | | 1392 |
| | | gag
Glu | | | | | | | | | 1440 |
| | | aga
Arg | | | | | | | | | 1488 |
| | | gaa
Glu | | Arg | | | Glu | | | | 1536 |
| | | gaa
Glu
515 | Lys | | | Arg | | | | | 1584 |

| cag
Gln | aga
Arg
530 | Gln | gaa
Glu | gaa
Glu | gaa
Glu | ggg
Gly
535 | aga
Arg | gaa
Glu | aga
Arg | caa
Gln | aga
Arg
540 | Gly | gag
Glu | gag
Glu | aga
Arg | 1632 |
|-------------------|----------------------------------|------------|----------------|------------|-------------------|-------------------|------------|------------|------------|-------------------|-------------------|------------|------------|------------|-------------------|------|
| gaa
Glu
545 | gag
Glu | aga
Arg | gag
Glu | aga
Arg | gaa
Glu
550 | ttt
Phe | caa
Gln | cag
Gln | cgc
Arg | gag
Glu
555 | cgg
Arg | gag
Glu | ctg
Leu | aag
Lys | aca
Thr
560 | 1680 |
| | cta
Leu | | | | | | | | | | | | | | | 1728 |
| | aag
Lys | | | | | | | | | | | | | | | 1776 |
| | aaa
Lys | | | | | | | | | | | | | | | 1824 |
| | aga
Arg
610 | | cg | | | | | | | | | | | • | | 1835 |
| <213
<213 | 0> 81
1> 61
2> PF
3> To | . 1
RT | .asma | gor | ıdii | | | | | | | | | | | |
| | 0> 81
Ile | - | G) v | Asn | Gln | Tur | Ser | Cvs | Len | Gln | Ara | Gly | Nlα | C) | C1 | |
| 1 | 110 | | O _I | 5 | 01 | - 7 - | 501 | Cys | 10 | GIII | nrg | GIY | Ala | 15 | Gly | |
| Asp | Lys | Glu | Thr
20 | Ala | Thr | Glu | Arg | Glu
25 | Glu | Arg | Asn | Arg | Glu
30 | Asp | Ala | |
| Pro | Ser | Phe
35 | Leu | Glu | Gly | Gly | Leu
40 | Gly | Asp | Asp | Glu | Thr
45 | Glu | Arg | Ala | |
| Lys | Gln
50 | Ala | Ser | Glu | Leu | Pro
55 | Ala | Ser | Leu | Cys | Ser
60 | Phe | Ala | Ala | Ala | |
| Arg
65 | Arg | Gly | Ala | Ser | Arg
70 | Ala | Glu | Lys | Thr | Gly
75 | Ala | Lys | Gly | Glu | Glu
80 | |

Ala Arg Glu Lys Glu Val Ser Phe Gly Glu Asp Ser Gly Leu Ser Arg

Gln Val Asp Met Asp Ser Ser Gln Glu Ser Val Asn Glu Gly Glu Pro Leu His Asp Arg Ala Ala Gly Glu Asp Ala Glu Gly Gly Ala Glu Ala Asn Asp Gly Asp Arg Glu Gly Asp Glu Lys Glu Thr Arg Asp Val Glu Asp Glu Gly Glu Thr Arg Arg Ser Ser Ser Phe Ala Glu Gln Thr Gly Asn Glu Arg Thr Glu Met Arg Thr Arg His Gly Gly Asp Glu Gly Trp Thr Ser Lys Ser Asn Arg Phe Ala Phe Ala Cys Pro Arg Phe Ser Lys Ser Asp Val Cys Cys Ser Pro Gln Ala Arg Leu Ser Leu Pro Glu Gln Ser Leu Gly Ser Ser Pro Ser Ser Pro Ile Ser Val Thr Asn Asp Val Tyr Ala Leu Phe Asp Ser Ser Ala Ser Pro Leu His Ala Gly Glu Leu Ser Ser Leu Pro Gly Ala Val Ser Ala Ser Glu Arg Leu Leu Thr Ala Pro Ala Glu Ile Gly Pro Ser Ala Ser Ser Ala Cys Leu Ser Val Ser Cys Gly Pro Gly Glu Met Ser Pro Thr Ala Asp Thr Thr Arg His Asp Ala Glu Glu Arg Glu Arg Arg Ala Glu Glu Glu Lys Glu Arg Glu Arg Gln Glu Glu Glu Arg Glu Arg Arg Arg Val Glu Glu Glu Lys Glu Arg Glu Arg Gln Glu Glu Glu Arg Glu Arg Arg Arg Val Glu Glu Glu Lys Ala Arg Gln Arg Glu Glu Asp Glu Arg Glu Arg Arg

Arg Val Glu Glu Glu Lys Ala Arg Gln Arg Glu Glu Glu Glu Arg Glu Arg Arg Arg Val Glu Glu Glu Lys Ala Arg Gln Arg Glu Glu Glu Glu Glu Arg Glu Arg Arg Val Glu Glu Glu Lys Ala Arg Gln Arg Glu Glu Glu Glu Arg Glu Arg Arg Arg Val Glu Glu Lys Ala Arg Gln Arg Glu Glu Glu Glu Arg Glu Gly Arg Arg Val Glu Glu Lys Ala Arg Gln Arg Glu Glu Glu Glu Arg Glu Gly Arg Arg Val Glu Glu Glu Lys Ala Arg Gln Arg Glu Glu Glu Glu Arg Glu Arg Arg Arg Val Glu Glu Glu Lys Glu Arg Glu Arg Glu Glu Glu Glu Arg Glu Arg Arg Arg Val Glu Glu Glu Lys Glu Arg Glu Arg Glu Glu Glu Glu Glu Arg Glu Arg Arg Arg Val Glu Glu Glu Lys Glu Arg Glu Arg Gir. Glu Glu Glu Lys Arg Glu Arg Arg Val Glu Glu Glu Lys Al. Ar: Gin Arg Gin Glu Glu Gly Arg Glu Arg Gin Arg Gly Glu Glu Arg Glu Glu Arg Glu Arg Glu Phe Gln Gln Arg Glu Arg Glu Leu Lvc Thr Arg Leu Val Glu Leu Gln Arg Glu His Ala Glu Ser Val Glu Thr Tr; Met Lys Glu Gln Gly Glu Arg Glu Arg His Leu Thr Gln Asp Trp Glu Arg Lys Leu His Ala Phe Glu Glu Gln Ser Arg Thr Val Leu Leu Glm

Glu Arg Ser 610

<210> 82

<211> 604

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(336)

<400> 82

ccg atg caa ttt gtc tct cct tcc cct ttt gtg caa tcc gac tcc ccc 48
Pro Met Gln Phe Val Ser Pro Ser Pro Phe Val Gln Ser Asp Ser Pro

1 5 10 15

tct tcg ccc ttc gca caa tcg gct tca cct cct tcc gag tac caa 96 Ser Ser Pro Phe Ala Gln Ser Ala Ser Pro Pro Pro Ser Glu Tyr Gln 20 25 30

gac tct ctt tcc ctt cct ttg gca gaa tcc gtc tcg tcg ctt cct ttg 144
Asp Ser Leu Ser Leu Pro Leu Ala Glu Ser Val Ser Ser Leu Pro Leu
35 40 45

gcg aaa cag gct tct cct ctt cac ttg aca caa cac cct tct ccc ctt 192
Ala Lys Gln Ala Ser Pro Leu His Leu Thr Gln His Pro Ser Pro Leu
50 55 60

cta tgg aca cag cgg gcc tct cca tct cct ttc ttg gtt caa cgg gat 240 Leu Trp Thr Gln Arg Ala Ser Pro Ser Pro Phe Leu Val Gln Arg Asp 65 70 75 80

tcg tca cct cct tct gcg tca atg cgg ctt tct gct cgt cct ttg gca 288 Ser Ser Pro Pro Ser Ala Ser Met Arg Leu Ser Ala Arg Pro Leu Ala 85 90 95

aaa cat gtc tct ccc ctt ctc cgg gca aaa cag gct tct cct ttt cca 336 Lys His Val Ser Pro Leu Leu Arg Ala Lys Gln Ala Ser Pro Phe Pro 100 105 110

tagaaccage agegggeete tecateteet ttettggtee aeegggttte gtteteettt 396

catcogtcaa tgcaggtttc atctcgtcct ttggggaaac atgtccctcc ccttctccgg 456

gcaaaacagg cttctccttt tccatagaac cagcagcggg cctctccatc tccgttggtg 516

gtccaccggg tttcgttctc ttttcatctg tcaatgcagg tttcgtctcg tgctttggca 576
aaacatgtcc ctcccttct ccggggtg 604

<210> 83

<211> 112

<212> PRT

<213> Toxoplasma gondii

<400> 83

Pro Met Gln Phe Val Ser Pro Ser Pro Phe Val Gln Ser Asp Ser Pro 1 5 10 15

Ser Ser Pro Phe Ala Gln Ser Ala Ser Pro Pro Pro Ser Glu Tyr Gln
20 25 30

Asp Ser Leu Ser Leu Pro Leu Ala Glu Ser Val Ser Ser Leu Pro Leu 35 40 45

Ala Lys Gln Ala Ser Pro Leu His Leu Thr Gln His Pro Ser Pro Leu 50 55 60

Leu Trp Thr Gln Arg Ala Ser Pro Ser Pro Phe Leu Val Gln Arg Asp
65 70 75 80

Ser Ser Pro Pro Ser Ala Ser Met Arg Leu Ser Ala Arg Pro Leu Ala 85 90 95

Lys His Val Ser Pro Leu Leu Arg Ala Lys Gln Ala Ser Pro Phe Pro 100 105 110

<210> 84

<211> 549

<212> DNA

<213> Toxoplasma gondii

<400> 84

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ccattettegg caaaacaage ttgteette ceatagaace ageagegge etetecateg 300 ccattettegg tecacegggt ttegteete ttteateegt caatgeaggt ttegtetegt 360 cetttggcaa aacatgtete teecettete egggcaaaace aggettetee ttttecatag 420 aaceageage gggeetetee ateteette ttggteeace gggtttegtt etettetat 480 cegteaatge aggtttegte teegteettag geaaaacatg teteteeet teetegggca 540 acacaageg

<210> 85

<211> 270

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(270)

<400> 85

cgg acg gat gaa cac tgg tgc atc atg aag gat att ggc tac aug qqc 48
Arg Thr Asp Glu His Trp Cys Ile Met Lys Asp Ile Gly Tyr Lys Gly
1 5 10 15

aca gac tcg aag tca aca aaa gca aac tca gcg gca gag tgc cun can 96 Thr Asp Ser Lys Ser Thr Lys Ala Asn Ser Ala Ala Glu Cys Glm Glm 20 25 30

atg tgc ctc aac gat gag agg tgt gac ttt ttc acg tgg caa can gc: 144 Met Cys Leu Asn Asp Glu Arg Cys Asp Phe Phe Thr Trp Gln Gln Ala 35 40 45

ggc aag cat tgt tgg ttt aag gct ggg gcg tcc act gcc tca aca ana 192 Gly Lys His Cys Trp Phe Lys Ala Gly Ala Ser Thr Ala Ser Thr Lys 50 55 60

tac aat cgg gct ggc gac tat tot gca cca aaa cac tgc ggc ct: cog 240 Tyr Asn Arg Ala Gly Asp Tyr Ser Ala Pro Lys His Cys Gly Leu Fro 65 70 75 80

270

acc aca tgt gtc aag gag cgg acc aag tcg
Thr Thr Cys Val Lys Glu Arg Thr Lys Ser
85 90

<210> 86

<211> 90

<212> PRT

<213> Toxoplasma gondii

<400> 86

Arg Thr Asp Glu His Trp Cys Ile Met Lys Asp Ile Gly Tyr Lys Gly
1 5 10 15

Thr Asp Ser Lys Ser Thr Lys Ala Asn Ser Ala Ala Glu Cys Gln Gln 20 25 30

Met Cys Leu Asn Asp Glu Arg Cys Asp Phe Phe Thr Trp Gln Gln Ala 35 40 45

Gly Lys His Cys Trp Phe Lys Ala Gly Ala Ser Thr Ala Ser Thr Lys 50 55 60

Tyr Asn Arg Ala Gly Asp Tyr Ser Ala Pro Lys His Cys Gly Leu Pro
65 70 75 80

Thr Thr Cys Val Lys Glu Arg Thr Lys Ser 85 90

<210> 87

<211> 306

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(306)

<400> 87

cgg cgg caa caa atg ggc cct gtt cga gcc cct gac ctc caa ttc aac 48
Arg Arg Gln Gln Met Gly Pro Val Arg Ala Pro Asp Leu Gln Phe Asn
1 5 10 15

cag tcg cca ctg ctc ccc cac aac ctc ggc cct gcc cac gtt ccc atg 96
Gln Ser Pro Leu Leu Pro His Asn Leu Gly Pro Ala His Val Pro Met
20 25 30

gga ggt ctc ccg tcg cat cct cat atc tcg gac ttt cat aac tca tcg 144 Gly Gly Leu Pro Ser His Pro His Ile Ser Asp Phe His Asn Ser Ser 35 40 45

gag teg ege eeg caa cat eeg etg ett gee age ggg ete gea teg aga 192

Glu Ser Arg Pro Gln His Pro Leu Leu Ala Ser Gly Leu Ala Ser Arg 50 55 60

ctc gga cag ggc ctg acg ccc cag gag aga cag ttc gtg ctc tct caa 240 Leu Gly Gln Gly Leu Thr Pro Gln Glu Arg Gln Phe Val Leu Ser Gln 65 70 75 80

cag tct ggc gga tcg acc tcg ttc ctg ctg cct gcg ttg ccg tct ctc $$ 288 Gln Ser Gly Gly Ser Thr Ser Phe Leu Leu Pro Ala Leu Pro Ser Leu $$ 85 $$ 90 $$ 95

tca gag aac ctc tcc gcg
Ser Glu Asn Leu Ser Ala
100

<210> 88

<211> 102

<212> PRT

<213> Toxoplasma gondii

<400> 88

Arg Arg Gln Gln Met Gly Pro Val Arg Ala Pro Asp Leu Gln Phe Asn.
1 5 10 15

Gln Ser Pro Leu Leu Pro His Asn Leu Gly Pro Ala His Val Pro Met
20 25 30

Gly Gly Leu Pro Ser His Pro His Ile Ser Asp Phe His Asn Ser Ser 35 40 45

Glu Ser Arg Pro Gln His Pro Leu Leu Ala Ser Gly Leu Ala Ser Arg 50 55 60

Leu Gly Gln Gly Leu Thr Pro Gln Glu Arg Gln Phe Val Leu Ser Gln 65 70 75 80

Gln Ser Gly Gly Ser Thr Ser Phe Leu Leu Pro Ala Leu Pro Ser Leu 85 90 95

Ser Glu Asn Leu Ser Ala 100

<210> 89

<211> 804

<212> DNA

<213> Toxoplasma gondii

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165 170 175

acc tcg cag cct cca ctt gag cgt caa aag tcg cag cgc ctc gcg caa 576 Thr Ser Gln Pro Pro Leu Glu Arg Gln Lys Ser Gln Arg Leu Ala Gln 180 185 acc gag cct gtg cag aaa ctc aag aca tcc tgg ttg gag cct cct caa Thr Glu Pro Val Gln Lys Leu Lys Thr Ser Trp Leu Glu Pro Pro Gln 200 195 672 gag gtc gaa agt gga cat gga gtc gct gaa ggc gac gat ctc agc gtt Glu Val Glu Ser Gly His Gly Val Ala Glu Gly Asp Asp Leu Ser Val 220 210 215 gca gca gcc gag tat cac gtc cca gaa acg gaa gat gga aaa ccc agc 720 Ala Ala Ala Glu Tyr His Val Pro Glu Thr Glu Asp Gly Lys Pro Ser 230 225 ttc aaa cct agc gac ccc cgc gtg tgg aat cgc gag tgg atc cac cga 768 Phe Lys Pro Ser Asp Pro Arg Val Trp Asn Arg Glu Trp Ile His Arg 250 245 804 agg ata cat aac ccc gtc ctc agt cgc tcg aac cgg Arg Ile His Asn Pro Val Leu Ser Arg Ser Asn Arg 265 260

<210> 90

<211> 268

<212> PRT

<213> Toxoplasma gondii

<400> 90

Arg Gly Gly Ile Ser Val Pro Thr Leu Ser Ile Met Asn Gln Ser Thr 1 5 10 15

Ile Val Ala Thr Ser Val Val Ala Pro Gln Ser Ala Val Ser Leu Ser 20 25 30

Arg Ala Pro Ser Arg Pro Gly Pro Ser Glu Ser Phe Gly Lys Gln Gln
35 40 45

Glu Ser Arg Pro Gly Val Ser Gly Ala Gly Leu Ala Glu Ser Lys Arg
50 55 60

Val Pro Ser Leu Thr Gln Pro Ser Leu Glu Arg Ser Val Thr Ile Ser 65 70 75 80

Arg Arg Lys Ile Asp Ala Val Gly Met Ser Leu Val Pro Lys Leu Asp 85 90 95

Arg Thr Thr Ser Leu Ala Ala Lys Glu Glu Lys Phe Ser Ser Ile 100 105 110

Asp Lys Ile Val Ser Lys Pro Thr. His Ser Phe Gly Glu Ser Ser Lys
115 120 125

Leu Pro Ala Gly Ile Met Lys Ala Lys Ser Met Phe Pro Ser Gln Thr 130 135 140

Leu Ser Ala Pro Trp Asn Ala Pro Ala Arg Cys Ala Arg Lys Asp Ser 145 150 155 160

Phe Gly Thr Lys Ala Trp Ile Glu Lys Leu Gln Arg Glu Thr Thr Asp 165 170 175

Thr Ser Gln Pro Pro Leu Glu Arg Gln Lys Ser Gln Arg Leu Ala Gln 180 185 190

Thr Glu Pro Val Gln Lys Leu Lys Thr Ser Trp Leu Glu Pro Pro Gln
195 200 205

Glu Val Glu Ser Gly His Gly Val Ala Glu Gly Asp Asp Leu Ser Val 210 215 220

Ala Ala Glu Tyr His Val Pro Glu Thr Glu Asp Gly Lys Pro Ser 225 230 235 240

Phe Lys Pro Ser Asp Pro Arg Val Trp Asn Arg Glu Trp Ile His Arg 245 250 255

Arg Ile His Asn Pro Val Leu Ser Arg Ser Asn Arg 260 265

<210> 91

<211> 867

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(867)

<400> 91

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| Arg
1 | Asp | Pro | Ala | Gly
5 | Lys | Ala | Val | Lys | Lys
10 | Ala | Ala | Thr | Gly | Ile
15 | Pro | |
|----------|-----|-----|-----|----------|-----|-----|-----|-----|-----------|-----|-----|-------------------|-----|-----------|-----|-----|
| - | | | | | | | | | | | | act
Thr | | | | 96 |
| _ | | | - | - | | - | - | - | _ | | - | ggc
Gly
45 | - | | - | 144 |
| _ | - | - | | - | - | | | | | | | ccc
Pro | | | | 192 |
| _ | _ | | | | | | | | | | | gca
Ala | | | | 240 |
| | _ | - | | | | | | | | | | aag
Lys | | | | 288 |
| | | | | | | | | | | | | gtg
Val | | | | 336 |
| | | | | | | | | | | | | tcc
Ser
125 | | | | 384 |
| _ | | | | | | | | | | | | gca
Ala | | | | 432 |
| | | - | | - | | | | | | | | aag
Lys | | | | 480 |
| - | - | | | | | | | | | | | cca
Pro | | | | 528 |
| | | | | | | | | | | | | gca
Ala | | | | 576 |
| ggc | aag | gca | gtg | aag | aag | cca | gtt | gtc | agc | gtg | cca | aag | cct | gca | acg | 624 |

Gly Lys Ala Val Lys Lys Pro Val Val Ser Val Pro Lys Pro Ala Thr 200 ctc gat ggc aag gcg gtg aga aag cca gtt gtc ggc gtg cca aag ccc 672 Leu Asp Gly Lys Ala Val Arg Lys Pro Val Val Gly Val Pro Lys Pro 210 215 gca gct ccc gat ggt aag gcg gtg aaa aag cca gtt gtc ggc gtg cca 720 Ala Ala Pro Asp Gly Lys Ala Val Lys Lys Pro Val Val Gly Val Pro 230 aag oot goa got ooa gat gac acg gga atc aac aag gog acc ott gto Lys Pro Ala Ala Pro Asp Asp Thr Gly Ile Asn Lys Ala Thr Leu Val 250 245 255 acg cgg aaa cct gag gct cca gac gtg aag gta gtc aag aag gca acc Thr Arg Lys Pro Glu Ala Pro Asp Val Lys Val Lys Lys Ala Thr 260 265 gta gtt gtg cca aaa cct gaa gcg cca gat ata aag gta atg acg gat Val Val Pro Lys Pro Glu Ala Pro Asp Ile Lys Val Met Thr Asp 275 280 285 ccg 867 Pro <210> 92 <211> 289 <212> PRT <213> Toxoplasma gondii <400> 92 Arg Asp Pro Ala Gly Lys Ala Val Lys Lys Ala Ala Thr Gly Ile Pro Lys Pro Ala Ala Pro Gly Gly Lys Ala Val Lys Val Thr Pro Val Ala 25 Arg Lys Pro Val Ala Pro Lys Ala Ala Pro Asp Gly Lys Ala Val 35 40 45 Lys Lys Ala Thr Val Val Val Pro Lys Pro Ala Ala Pro Ser Gly Lys 50 55 Ala Val Lys Lys Pro Val Val Ser Val Pro Lys Pro Ala Thr Leu Gly 70 75 65

Gly Lys Ala Val Lys Lys Pro Ala Ala Gly Val Pro Lys Pro Ala Ala 85 90 95

- Pro Asp Gly Lys Ala Val Arg Lys Pro Val Val Gly Val Pro Lys Pro 100 105 110
- Ala Ala Pro Asp Gly Lys Ala Ala Lys Lys Pro Ala Ser Gly Val Pro 115 120 125
- Lys Pro Ala Asp Pro Ala Gly Lys Ala Val Lys Lys Ala Ala Thr Gly 130 135 140
- Val Ala Arg Lys Pro Val Ala Pro Lys Ala Ala Ala Pro Asp Gly Lys 165 170 175
- Ala Val Lys Lys Ala Thr Val Val Val Pro Lys Pro Ala Ala Pro Ser 180 185 190
- Gly Lys Ala Val Lys Lys Pro Val Val Ser Val Pro Lys Pro Ala Thr 195 200 205
- Leu Asp Gly Lys Ala Val Arg Lys Pro Val Val Gly Val Pro Lys Pro 210 215 220
- Ala Ala Pro Asp Gly Lys Ala Val Lys Lys Pro Val Val Gly Val Pro 225 230 235 240
- Lys Pro Ala Ala Pro Asp Asp Thr Gly Ile Asn Lys Ala Thr Leu Val 245 250 255
- Thr Arg Lys Pro Glu Ala Pro Asp Val Lys Val Val Lys Lys Ala Thr 260 265 270
- Val Val Pro Lys Pro Glu Ala Pro Asp Ile Lys Val Met Thr Asp 275 280 285

Pro

<210> 93

<211> 1434

<212> DNA

<213> Toxoplasma gondii

<221> CDS <222> (1)..(492) <400> 93 egg ett gig itg eee gga gaa ggg gag aga eat git itg eea aag gae Arg Leu Val Leu Pro Gly Glu Gly Glu Arg His Val Leu Pro Lys Asp. 10 gag acg aaa cot goa tig acg gat gaa aag aga acg aaa coo ggt aga 96 Glu Thr Lys Pro Ala Leu Thr Asp Glu Lys Arg Thr Lys Pro Gly Gly 20 25 cca agg aag gag atg gag agg ccc gct gct ggt tct atg gaa aag gad Pro Arg Lys Glu Met Glu Arg Pro Ala Ala Gly Ser Met Glu Lys Asp 35 aag oft git tig ood gga gaa ggg gag aga cat git tig ood aa + aa + aa + aaLys Leu Val Leu Pro Gly Glu Gly Glu Arg His Val Leu Pro Lys Asp 55 gag acg aaa cct gca ttg acg gag gaa aag aga acg aaa ccc qq: q:; Glu Thr Lys Pro Ala Leu Thr Glu Glu Lys Arg Thr Lys Pro Gly Way 65 70 cca cga acg gag atg gag agg ccc gct gct ggt tct atg qau $\alpha\alpha$ 1 α . 188 Pro Arg Thr Glu Met Glu Arg Pro Ala Ala Gly Ser Met Glu Ly: Arg 85 aag oot ggt ttg ooc gga gaa ggg gag aga oat gtt ttg oo $_{\rm col}$ $_{\rm col}$; $_{\rm col}$ 336 Lys Pro Gly Leu Pro Gly Glu Gly Glu Arg His Val Leu Pro Lys Ant 100 105 gag acg aaa cot goa ttg acg gag gaa aag aga acg aac ctq $q^{-1}:=1$ Glu Thr Lys Pro Ala Leu Thr Glu Glu Lys Arg Thr Asn Leu A. . A.; 115 120 caa gaa agg aga tgg aga goo ogo tgo tgg tto tto qaa aad dir are Gln Glu Arg Arg Trp Arg Ala Arg Cys Trp Phe Leu Glu Lyc Gla Acr. 140 130 135 ctg ttt ggc ccg gag aag ggg aga gac acg ctt cgc caa aqq ir; iii. Leu Phe Gly Pro Glu Lys Gly Arg Asp Thr Leu Arg Gln Arg T:: Arı 145 150 155 cga aag ccg cat tgacgcaaaa ggaggtgacg aatcccgttg aaccaagaau 532 Arg Lys Pro His

<220>

ggcgatggag aggcccgctg ctggttctat ggaaaaggaa aacctgtttc cccggagaag 592 gggagggaca tgttttgcca aagcacagac gaaacctgca ttgacagatg aaaagagaac 652 gaaacccggt ggaccaccaa cggagatgga gaggcccgct gctggtttta tggaaaagga 712 qaageetgtt ttgeeeggag aaggggaggg acatgtttee ecaaaggaeg agatgaaace 772 tgcattgacg gatgaaaaga gaacgaaacc cggtggacca agaaaggaga tggagaggcc 832 cgctgctggt tttatggaaa aggagaagcc tgttttgccc ggagaagggg agagacatgt 892 tttgccaaag gacgagcaga aagccgcatt gacgcagaag gaggtgacga atcccgttga 952 accaagaaag gagatggaga ggcccgctgt gcccatagaa ggggagaagg gtgttgtgtc 1012 aagtgaagag gagaagcctg tttcgccaaa ggaagcgacg agacggattt tgccaaagga 1072 agggaaagag atttggtact cggaaggagg aggtgaagcc gattgtgcga agggcaaaga 1132 qqqqqaqacq gattqcacaa aaqqqqaaqq agaaacaaat tqcatcqaaq gaqqqqaaqa 1192 aacccgctgt accaaaggaa ggtgaggaaa gacccgctga accaacggaa ggcgaggaaa 1252 qqcccqttqq qccaaaqgaa qqcqaqgaaa qacccqttqt qccqqacqta qacaaqgaqa 1312 aacctgttgt gcctgaagga gacaaggaga aacctgttgt gccggaagga gacaaggatc 1372 accetgette tgecagagea ggatgaggag aaacaegeta catgggagaa agaaatgate 1432 1434 cg

<210> 94

<211> 164

<212> PRT

<213> Toxoplasma gondii

<400> 94

Arg Leu Val Leu Pro Gly Glu Gly Glu Arg His Val Leu Pro Lys Asp 1 5 10 15

Glu Thr Lys Pro Ala Leu Thr Asp Glu Lys Arg Thr Lys Pro Gly Gly
20 25 30

Pro Arg Lys Glu Met Glu Arg Pro Ala Ala Gly Ser Met Glu Lys Asp 35 40 45

Lys Leu Val Leu Pro Gly Glu Gly Glu Arg His Val Leu Pro Lys Asp
50 55 60

Glu Thr Lys Pro Ala Leu Thr Glu Glu Lys Arg Thr Lys Pro Gly Gly
65 70 75 80

Pro Arg Thr Glu Met Glu Arg Pro Ala Ala Gly Ser Met Glu Lys Asp 85 90 95

Lys Pro Gly Leu Pro Gly Glu Gly Glu Arg His Val Leu Pro Lys Asp 100 105 110

Glu Thr Lys Pro Ala Leu Thr Glu Glu Lys Arg Thr Asn Leu Ala Asp 115 120 125

Gln Glu Arg Arg Trp Arg Ala Arg Cys Trp Phe Leu Glu Lys Glu Asn 130 135 140

Leu Phe Gly Pro Glu Lys Gly Arg Asp Thr Leu Arg Gln Arg Thr Arg 145 150 155 160

Arg Lys Pro His

<210> 95

<211> 680

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(678)

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cag aaa ctt gtt ttg caa aag aca gag agg aaa cca gtt ttg cca gag 96 Gln Lys Leu Val Leu Gln Lys Thr Glu Arg Lys Pro Val Leu Pro Glu 20 25 30

gaa gac cag aaa ccg gtt tta cca gaa aca ggg gcg aaa cat gtt tta 144 Glu Asp Gln Lys Pro Val Leu Pro Glu Thr Gly Ala Lys His Val Leu 35 40 45

ccg gaa ata gcg acc gaa tcc act ttg acg cag aaa gag ctg aca aaa 192

PCT/US98/27137 WO 99/32633 Pro Glu Ile Ala Thr Glu Ser Thr Leu Thr Gln Lys Glu Leu Thr Lys 55

ccc qtt qaa aca aga cag gac atg agg ggg acc gct ggt tct atg gac 240 Pro Val Glu Thr Arg Gln Asp Met Arg Gly Thr Ala Gly Ser Met Asp 75 70 gag aag aag oot git tig ooc gga gaa tigg gag aga cat gio tig ooa 288 Glu Lys Lys Pro Val Leu Pro Gly Glu Trp Glu Arg His Val Leu Pro 90 85 aaa gac gag acg aaa cct gca ttg acg gag gaa aag aga acg aaa ccc 336 Lys Asp Glu Thr Lys Pro Ala Leu Thr Glu Glu Lys Arg Thr Lys Pro 105 100 384 gtt gaa cca aga aag gag atg gag agg ccc gct cgc ccc atg gaa gag Val Glu Pro Arg Lys Glu Met Glu Arg Pro Ala Arg Pro Met Glu Glu 125 120 115 gag aag oot gtt tta ooc gga gaa ggg gag aga oat gtt tta oou aun 432 Glu Lys Pro Val Leu Pro Gly Glu Gly Glu Arg His Val Leu Fre Lys 135 130 gac ggg atg aaa oot goa ttg acg gat gaa aag aga acg aaa coo gqt 480 Asp Gly Met Lys Pro Ala Leu Thr Asp Glu Lys Arg Thr Lys Fre Gly 155 150 145 gga cca agg aag gag atg gag agg ccc gct gct ggt tct atg qua dag 528 Gly Pro Arg Lys Glu Met Glu Arg Pro Ala Ala Gly Ser Met Glu Lys 1 ... 170 165 gac aag off gtg ttg coo gga gaa ggg gag aga cat gtf ttg cot aag 576 Asp Lys Leu Val Leu Pro Gly Glu Gly Glu Arg His Val Leu Pro Lys 185 180 gac gag acg aaa cot goa ttg acg gat gaa aag aga acg aau coo qq: 624 Asp Glu Thr Lys Pro Ala Leu Thr Asp Glu Lys Arg Thr Lys tro Gly 205 195 200 gga cca aga aay gcg atg gag agg ccc gct gct ggt tct atg dan and 672 Gly Pro Arg Lys Ala Met Glu Arg Pro Ala Ala Gly Ser Met Glu Lys 220 215 210

680 gac aag cg Asp Lys 225

<210> 96

<211> 226

<212> PRT

<213> Toxoplasma gondii

<400> 96

Arg Pro Arg Ala Gly Arg Glu Gln Pro Ala Val Pro Arg Gln Glu Glu
1 5 10 15

Gln Lys Leu Val Leu Gln Lys Thr Glu Arg Lys Pro Val Leu Pro Glu 20 25 30

Glu Asp Gln Lys Pro Val Leu Pro Glu Thr Gly Ala Lys His Val Leu 35 40 45

Pro Glu Ile Ala Thr Glu Ser Thr Leu Thr Gln Lys Glu Leu Thr Lys 50 55 60

Pro Val Glu Thr Arg Gln Asp Met Arg Gly Thr Ala Gly Ser Met Asp 65 70 75 80

Glu Lys Lys Pro Val Leu Pro Gly Glu Trp Glu Arg His Val Leu Pro 85 90 95

Lys Asp Glu Thr Lys Pro Ala Leu Thr Glu Glu Lys Arg Thr Lys Pro 100 105 110

Val Glu Pro Arg Lys Glu Met Glu Arg Pro Ala Arg Pro Met Glu Glu 115 120 125

Glu Lys Pro Val Leu Pro Gly Glu Gly Glu Arq His Val Leu Pro Lys 130 135 140

Asp Gly Met Lys Pro Ala Leu Thr Asp Glu Lys Arg Thr Lys Pro Gly 145 150 155 160

Gly Pro Arg Lys Glu Met Glu Arg Pro Ala Ala Gly Ser Met Glu Lys 165 170 175

Asp Lys Leu Val Leu Pro Gly Glu Gly Glu Arg His Val Leu Pro Lys 180 185 190

Asp Glu Thr Lys Pro Ala Leu Thr Asp Glu Lys Arg Thr Lys Pro Gly
195 200 205

Gly Pro Arg Lys Ala Met Glu Arg Pro Ala Ala Gly Ser Met Glu Lys 210 220 .

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Asp Lys
225
<210> 97
<211> 296
<212> DNA
<213> Toxoplasma gondii
<220>
<221> CDS
<222> (1)..(294)
<400> 97
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Pro Val Asp Val Asp Asp Pro Arg Gly Cys Ser Gln Gln Ser Gly Asp
                                                          15
                                      10
                   5
ace aga gae age age cee gee aca cet ggt ggt egg eeg get ggt
                                                                   96
Thr Arg Asp Ser Ser Pro Ala Thr Pro Gly Gly Arg Pro Ala Gly
                                  25
              20
ggg gca gga ggt gca gcg aca agc ccg aag gga cag gcc ttt gcc ccg
                                                                   144
Gly Ala Gly Gly Ala Ala Thr Ser Pro Lys Gly Gln Ala Phe Ala Pro
                                                  45
                              40
          35
egg gge ggt gaa ggg gag ata aag eee cag gag aca gga aac agt gga
                                                                   192
Arg Gly Glu Gly Glu Ile Lys Pro Gln Glu Thr Gly Asn Ser Gly
                          55
      50
 gac agc aag gcg gag gga aag gaa gca agt gga gac gcg aac act tcg
                                                                    240
 Asp Ser Lys Ala Glu Gly Lys Glu Ala Ser Gly Asp Ala Asn Thr Ser
                                          75
                      70
  65
 gaa gga aag cga ttg tcg ggc gaa gtg gac aag aca gcc gag gtg gag
                                                                    288
 Glu Gly Lys Arg Leu Ser Gly Glu Val Asp Lys Thr Ala Glu Val Glu
                                       90
                   85
                                                                    296
 aca gcc gg
 Thr Ala
 <210> 98
  <211> 98
  <212> PRT
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<400> 98

<213> Toxoplasma gondii

Pro Val Asp Val Asp Pro Arg Gly Cys Ser Gln Gln Ser Gly Asp
1 5 10 15

Thr Arg Asp Ser Ser Pro Ala Thr Pro Gly Gly Arg Pro Ala Gly
20 25 30

Gly Ala Gly Gly Ala Ala Thr Ser Pro Lys Gly Gln Ala Phe Ala Pro 35 40 45

Arg Gly Glu Glu Glu Ile Lys Pro Gln Glu Thr Gly Asn Ser Gly 50 55 60

Asp Ser Lys Ala Glu Gly Lys Glu Ala Ser Gly Asp Ala Asn Thr Ser 65 70 75 80

Glu Gly Lys Arg Leu Ser Gly Glu Val Asp Lys Thr Ala Glu Val Glu 85 90 95

Thr Ala

<210> 99

<211> 723

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(159)

<400> 99

cga tcc tcc cga ggg acc gca gga agg ctc gcg tcc gaa gaa gac gac 48
Arg Ser Ser Arg Gly Thr Ala Gly Arg Leu Ala Ser Glu Glu Asp Asp
1 5 10 15

aga gaa gac ggg gaa gac gca ggc tct agg cgt cga gag aag gac ttc 144 Arg Glu Asp Gly Glu Asp Ala Gly Ser Arg Arg Arg Glu Lys Asp Phe 35 40 45

ttc cca gac acg act tgaatgcgta aaggcgtatt tttgtttccg atgaaaactc 199
Phe Pro Asp Thr Thr
50

<210> 100

<211> 53

<212> PRT

<213> Toxoplasma gondii

<400> 100

Arg Ser Ser Arg Gly Thr Ala Gly Arg Leu Ala Ser Glu Glu Asp Asp 1 5 10 15

Gly Asp Asn Glu Glu Glu Glu Glu Glu Glu Glu Arg Glu Arg Glu
20 25 30

Arg Glu Asp Gly Glu Asp Ala Gly Ser Arg Arg Glu Lys Asp Phe 35 40 45

Phe Pro Asp Thr Thr 50

<210> 101

<211> 270

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(270)

<400> 101

cgg aag ccg att gtg cga agg gca aag agg ggg aga cgg att gca caa 48 Arg Lys Pro Ile Val Arg Arg Ala Lys Arg Gly Arg Arg Ile Ala Gln 1 aag ggg aag gag aaa caa att gca tcg aag gag ggg aag aaa ccc gct . 96 Lys Gly Lys Glu Lys Gln Ile Ala Ser Lys Glu Gly Lys Lys Pro Ala 25 gta cca aag gaa ggt gag gaa aga ccc gct gaa cca acg gaa ggc gag Val Pro Lys Glu Gly Glu Glu Arg Pro Ala Glu Pro Thr Glu Gly Glu 35 40 gaa agg ccc gtt ggg cca aag gaa ggc gag gaa aga ccc gtt gtg ccg 192 Glu Arg Pro Val Gly Pro Lys Glu Gly Glu Glu Arg Pro Val Val Pro gac gta gac aag gag aaa cct gtt gtg cct gaa gga gac aag gag aaa Asp Val Asp Lys Glu Lys Pro Val Val Pro Glu Gly Asp Lys Glu Lys 65 70 75 cct gtt gtg ccg gaa gga gac aag gat ccg 270 Pro Val Val Pro Glu Gly Asp Lys Asp Pro 85

<210> 102

<211> 90

<212> PRT

<213> Toxoplasma gondii

<400> 102

Arg Lys Pro Ile Val Arg Arg Ala Lys Arg Gly Arg Arg Ile Ala Gln
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Lys Gly Lys Glu Lys Gln Ile Ala Ser Lys Glu Gly Lys Lys Pro Ala 20 25 30

Val Pro Lys Glu Gly Glu Glu Arg Pro Ala Glu Pro Thr Glu Gly Glu
35 40 45

Glu Arg Pro Val Gly Pro Lys Glu Gly Glu Glu Arg Pro Val Val Pro 50 55 60

Asp Val Asp Lys Glu Lys Pro Val Val Pro Glu Gly Asp Lys Glu Lys 65 70 75 80

Pro Val Val Pro Glu Gly Asp Lys Asp Pro 85 90

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<210> 103
<211> 503
<212> DNA
<213> Toxoplasma gondii
<220>
<221> CDS
<222> (1)..(186)
<400> 103
cgg cat ctc tgg tgc gtg cgc gag aga tcc ccg caa cga gaa aga tgg
                                                               48
Arg His Leu Trp Cys Val Arg Glu Arg Ser Pro Gln Arg Glu Arg Trp
                                   10
                 5
 1
age tte gte teg tte teg ett tte tte tet tte cag tte ttt tte age
                                                               96
Ser Phe Val Ser Phe Ser Leu Phe Phe Ser Phe Gln Phe Phe Ser
                               25
aag caa gtc tcg cgc ctc cct cgt ccg agc agc gtc act gca ctg tgg
Lys Gln Val Ser Arg Leu Pro Arg Pro Ser Ser Val Thr Ala Leu Trp
                            40
        35
                                                               186
Ala Ile Ser Arg Lys Lys Ala Lys Lys Arg Asp Asp Gly Arg
                                           60
                        55
     50
taatggcgcg aaaatctatc ccaaaaacac atatatgcct tatggcagtg agcgaagaga 246
gggaactgcc aacgccttgg cggaagcccg ttctccaaac gaggttgagg taccaaacct 306
gcatgcggag agaccaaggc aggttttgtc ttccgtcgct tccgtggatg cttttcgcac 366
gtatgcaaaa gagagaacgg gaccaagtgc aagaagttat agagcagtcc cgacgacaga 426
gacgcancta gaggccgagc aagaatcgtt tttttcttct cgtaagggaa acgcagtgca 486
                                                               503
tanaagcaaa agaccgg
<210> 104
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<211> 62

<212> PRT

<213> Toxoplasma gondii

<400> 104

Arg His Leu Trp Cys Val Arg Glu Arg Ser Pro Gln Arg Glu Arg Trp

| 1 | | | | 5 | | | | | 10 | | | | | 15 | | |
|--------------|----------------------------------|-----------|------------|-------|-------|-------------|-----------|-----------|------|------------|-----------|-----------|-----------|------|--------|-----|
| Ser | Phe | Vāl | Ser
20 | Phe | Ser | Leu | Phe | Phe
25 | Ser | Phe | Gln | Phe | Phe
30 | Phe | ·Ser | |
| Lys | Gln | Val
35 | Ser | Arg | Leu | Pro | Arg
40 | Pro | Ser | Ser | Val | Thr
45 | Ala | Leu | Trp | |
| Ala | Ile
50 | Ser | Arg | Lys | Lys | Ala
55 | Lys | Lys | Arg | Asp | Asp
60 | Gly | Arg | | | |
| <211
<212 |)> 10
.> 32
?> DN
3> To | 22
1A | lasma | a gor | ndii | | | | | | | | | | | |
| | > CE | | (219) | | | | | | | | | | | | | |
| < 4 0 0 |)> 10 | 5 | | | | | | | | | | | | | | |
| | | | | | | | | | | gct
Ala | | | | - | | 48 |
| | | | | | | | | | | tcg
Ser | | | | | _ | 96 |
| | | | | | | | | | | tgc
Cys | - | | | | | 144 |
| | | | | | | | | | | gcc
Ala | | | | - | | 192 |
| | | | ccg
Pro | | | | | | tgaa | aggaa | aat o | cacaç | gacat | | | 239 |
| acca | acct | itc (| ccgc | gtg | gc ta | aaag | gacco | g te | ctgt | gtat | gtad | agtt | ett t | ccaç | ggcgaa | 299 |
| agco | gaaq | gag a | acago | cgaaa | ac co | a 'a | | | | | | | | | | 322 |

PCT/US98/27137

<210> 106

WO 99/32633

<211> 73

<212> PRT

<213> Toxoplasma gondii

<400> 106

Arg Arg Asp Leu Arg Thr Ser Val Trp Asp Ala Arg Val Tyr Val His

1 5 10 15

Leu Ala Gly Gly Gln Arg Arg Cys Asn Glu Ser Arg Gly Met Glu Glu 20 25 30

Ala Arg Lys Arg Arg Cys Leu Ala Met Arg Cys Gln Trp Thr Xaa Ser 35 40 45

Ala Leu Asp Trp Arg Glu Ser Trp Lys Asn Ala Glu Thr Ala Ser His
50 55 60

Val Thr Phe Pro Thr Lys Arg Pro Pro 65 70

<210> 107

<211> 390

<212> DNA

<213> Toxoplasma gondii

<220>

 $\langle 223 \rangle$ N = unknown at 104

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 $\langle 223 \rangle$ Xaa = unknown at 35

<220>

<221> CDS

<222> (1)..(201)

<400> 107

cgg cga atc ccc cag gaa ttg ttg aaa cag agt ctc aga ttc tac gga 48
Arg Arg Ile Pro Gln Glu Leu Leu Lys Gln Ser Leu Arg Phe Tyr Gly
1 5 10 15

ctc cga ggg cct ctg ctt gcc cgc cct gtg cac agg cgt cag cac gtg 96
Leu Arg Gly Pro Leu Leu Ala Arg Pro Val His Arg Arg Gln His Val
20 25 30

gtt ctc ana gaa aaa gtt ggt aag tgg aag tgg tgg agc caa gaa aaa 144 Val Leu Xaa Glu Lys Val Gly Lys Trp Lys Trp Trp Ser Gln Glu Lys 35 40 45

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ctc aac tct tct tgt ttt ccg gag aat ttt cct ggt gtt caa ttc cac
Leu Asn Ser Ser Cys Phe Pro Glu Asn Phe Pro Gly Val Gln Phe His
     50
                         55
                                           . 60
ggt tot gga tagtotttgt tgtattaaaa cacatotaga aggactgaga
                                                                   241
Gly Ser Gly
65
cqttqtcqqt aqttqaatta cagacacttc qttttccagc qtcaqcttqc atqcccqtcc 301
cctgtttctg gaacacaagc tttgagaagg aaacgagaca gagaacgacg aaggaagtga 361
agcaaatcct ctgacggatt tccattcgg
                                                                   390
<210> 108
<211> 67
<212> PRT
<213> Toxoplasma gondii ...
<400> 108
Arg Arg Ile Pro Gln Glu Leu Lys Gln Ser Leu Arg Phe Tyr Gly
                  5
                                     10
Leu Arg Gly Pro Leu Leu Ala Arg Pro Val His Arg Arg Gln His Val
             20
                                 25
Val Leu Xaa Glu Lys Val Gly Lys Trp Lys Trp Trp Ser Gln Glu Lys
                             40
Leu Asn Ser Ser Cys Phe Pro Glu Asn Phe Pro Gly Val Gln Phe His
                         55
Gly Ser Gly
 65
<210> 109
<211> 699
<212> DNA
<213> Toxoplasma gondii
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<221> CDS
<222> (1)..(699)
<400> 109
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		gtc Val														48
		ccg Pro														96
		tgc Cys 35														144
		gag Glu													=	192
		aac Asn												_	_	240
		tgc Cys														288
		aat Asn												_	_	336
		acc Thr 115									-			-	-	384
-	_	gaa Glu		_	_	-		_		_	_	-			-	432
	_	gcg Ala			-			-		-						480
-	-	gaa Glu	_		-	-	-	-	-	-	, ,		_	,	-	528
_		ccc Pro			-	_		-	_		7 -	-		-	-	576

age ggc tgg ggc cag ggc gac tgc agc aaa cca gtc gac aag tgc gaa 624

Ser Gly Trp Gly Gln Gly Asp Cys Ser Lys Pro Val Asp Lys Cys Glu
195 200 200 205 .

gac gtc agt tgc aac aac ggt tca tca tgc gac gcg gac tcc ggc aca 672

Asp Val Ser Cys Asn Asn Gly Ser Ser Cys Asp Ala Asp Ser Gly Thr
210 215 220 699

Cys Ile Cys Pro Pro Gly Phe Gly Asp
230 624

<210> 110

<211> 233

<212> PRT

<213> Toxoplasma gondii

<400> 110

Pro Cys Val Cys Glu Glu Lys Cys Lys Thr Gly Pro Asn Cys Asp Gln
1 5 10 15

His Lys Pro Glu Cys Cys Gly Ser Asn Asp Asp Cys His Gln Pro Gln
20 25 30

Gly Tyr Cys Lys Met Asp Met Ser Thr Cys Ile Cys Arg Pro Gly Phe 35 40 45

Thr Gly Glu Asn Cys Gly Thr Arg Glu Asp Leu Cys Ala Gly Val Thr 50 55 60

Cys Lys Asn Gly Gly Thr Cys Asp Ser Val Thr Gly Leu Cys Gln Cys
65 70 75 80

Asp Ala Cys His Gly Gly Lys Thr Cys Glu Ile Thr Lys Glu His Cys 85 90 95

Cys Ile Asn Asp Ser Asp Cys Asn Gly His Gly Thr Cys Asn Thr Ser 100 105 110

Asn Asn Thr Cys Asn Cys Glu Ala Gly Phe Ala Gly Thr Asn Cys Ser 115 120 125

Ser Ser Glu Gly Lys Cys Ser Gly Lys Thr Cys Leu Ser Gly His Cys 130 135 140

Arg Cys Glu Thr Leu Val Lys Asp Cys Cys Val Val Asn Asp Thr Cys 170 165 Lys Phe Pro Asn Gly Val Cys Thr Asp Ser Asn Arg Cys Glu Cys Gln 185 180 Ser Gly Trp Gly Gln Gly Asp Cys Ser Lys Pro Val Asp Lys Cys Glu 200 205 195 Asp Val Ser Cys Asn Asn Gly Ser Ser Cys Asp Ala Asp Ser Gly Thr 215 220 210 Cys Ile Cys Pro Pro Gly Phe Gly Asp 230 225 <210> 111 <211> 419 <212> DNA <213> Toxoplasma gondii <220> <221> CDS <222> (1)..(417) <400> 111 gag atg age gee eea gat agg caa aca gga aag ett tee gat tta eeq Glu Met Ser Ala Pro Asp Arg Gln Thr Gly Lys Leu Ser Asp Leu Fre 10 15 5 1 cca ttt get gag etg eca eag etg gea gaa ata eca aag ete tee dan Pro Phe Ala Glu Leu Pro Gln Leu Ala Glu Ile Pro Lys Leu 50: Glu 3ũ 20 ctt ccg aaa atc gcg gac atg ccg aaa ttt tcg gat atg ccc auq at: Leu Pro Lys Ile Ala Asp Met Pro Lys Phe Ser Asp Met Pro Lys Met 40 35 ged gag atg ded aag tta tea gat ata ded aag atg get gag at; 192 Ala Glu Met Pro Lys Leu Ser Asp Ile Pro Lys Met Ala Glu Met Fr 55 50 aag tta tca gat ata ccc aag atg gct gag atg ccc aag tta tea dat 240 Lys Leu Ser Asp Ile Pro Lys Met Ala Glu Met Pro Lys Leu Ser Asi 75 70 288 ata ccc aag atg gct gag atg ccc aag ttt tca gat ata ccc aag at :

Ile Pro Lys Met Ala Glu Met Pro Lys Phe Ser Asp Ile Pro Lys Met 85 90 gct gag atg cca aag tta tca gat atg ccc. aga atg gct gac att cca 336 Ala Glu Met Pro Lys Leu Ser Asp Met Pro Arg Met Ala Asp Ile Pro 100 105 cag ttt cca gag atg cct agg atg gtt gac atg cct cag ttt cca gaa 384 Gln Phe Pro Glu Met Pro Arg Met Val Asp Met Pro Gln Phe Pro Glu 115 120 atc ccc agg atg gct gat atg ccg caa ttt ccg cg 419 Ile Pro Arg Met Ala Asp Met Pro Gln Phe Pro 130 135 <210> 112 <211> 139 <212> PRT <213> Toxoplasma gondii <400> 112 Glu Met Ser Ala Pro Asp Arg Gln Thr Gly Lys Leu Ser Asp Leu Pro 5 10 Pro Phe Ala Glu Leu Pro Gln Leu Ala Glu Ile Pro Lys Leu Ser Glu 20 25 Leu Pro Lys Ile Ala Asp Met Pro Lys Phe Ser Asp Met Pro Lys Met 40 Ala Glu Met Pro Lys Leu Ser Asp Ile Pro Lys Met Ala Glu Met Pro 55 Lys Leu Ser Asp Ile Pro Lys Met Ala Glu Met Pro Lys Leu Ser Asp 65 70 Ile Pro Lys Met Ala Glu Met Pro Lys Phe Ser Asp Ile Pro Lys Met 85 90 Ala Glu Met Pro Lys Leu Ser Asp Met Pro Arg Met Ala Asp Ile Pro 100 105 110 Gln Phe Pro Glu Met Pro Arg Met Val Asp Met Pro Gln Phe Pro Glu 115 120 125 Ile Pro Arg Met Ala Asp Met Pro Gln Phe Pro 135

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<212> DNA
<213> Toxoplasma gondii
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<221> CDS
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gac gaa gct ctt cct ctc ttt gga gca aac ggt gga acc tca gtt cgg
                                                                   48
Asp Glu Ala Leu Pro Leu Phe Gly Ala Asn Gly Gly Thr Ser Val Arg
                  5
                                     10
ctc tcc ctc gac cgc agc gtc ctg ctt gtt ctc gaa ccc gca gag ccc
                                                                   96
Leu Ser Leu Asp Arg Ser Val Leu Leu Val Leu Glu Pro Ala Glu Pro
           20
ctg cta tcc tct tgg ccc cac ccg ggg aga aga gac act ttt ctt gaa
                                                                   144
Leu Leu Ser Ser Trp Pro His Pro Gly Arg Arg Asp Thr Phe Leu Glu
                                                  45
                              40
         35
ggc gat ggc gcg ggc atc ccg tct cct tca tct cgg ccg agt cgc gcg
                                                                   192
Gly Asp Gly Ala Gly Ile Pro Ser Pro Ser Ser Arg Pro Ser Arg Ala
                                              60
     50
                         55
gcc gac cat tac acg aga ctc tcc acg att cgg tct ctt gcc agg gat
                                                                    240
Ala Asp His Tyr Thr Arg Leu Ser Thr Ile Arg Ser Leu Ala Arg Asp
gga gag gtc gac tcc gag ctg gcg ggg gga ccg cag gaa aga gaa agt
                                                                    288
Gly Glu Val Asp Ser Glu Leu Ala Gly Gly Pro Gln Glu Arg Glu Ser
                                                          95
                 85
                                      90
                                                                    303
gtc aga gtg gat ccg
Val Arg Val Asp Pro
            100
<210> 114
<211> 101
<212> PRT
<213> Toxoplasma gondii
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100

Asp Glu Ala Leu Pro Leu Phe Gly Ala Asn Gly Gly Thr Ser Val Arg

<400> 114

1 5 10 15

Leu Ser Leu Asp Arg Ser Val Leu Leu Val Leu Glu Pro Ala Glu Pro 20 25 30

Leu Leu Ser Ser Trp Pro His Pro Gly Arg Arg Asp Thr Phe Leu Glu 35 40 45

Gly Asp Gly Ala Gly Ile Pro Ser Pro Ser Ser Arg Pro Ser Arg Ala 50 55 60

Ala Asp His Tyr Thr Arg Leu Ser Thr Ile Arg Ser Leu Ala Arg Asp
65 70 75 80

Gly Glu Val Asp Ser Glu Leu Ala Gly Gly Pro Gln Glu Arg Glu Ser 85 90 95

Val Arg Val Asp Pro 100

<210> 115

<211> 696

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(696)

<400> 115

cgc ggt aac gaa aaa aca tgc tca gat gcc aag cat cca gtg tac atc 48
Arg Gly Asn Glu Lys Thr Cys Ser Asp Ala Lys His Pro Val Tyr Ile
1 5 10 15

aaa ctt ggc aaa ggg gaa cgc gag gcc gta ttc aag tgt ggc gac ggc 96 Lys Leu Gly Lys Gly Glu Arg Glu Ala Val Phe Lys Cys Gly Asp Gly 20 25 30

ctc act act ctt gag cca tcg cag aac aca gat aaa cca aaa ttc tgt 144 Leu Thr Thr Leu Glu Pro Ser Gln Asn Thr Asp Lys Pro Lys Phe Cys 35 40 45

gaa tcg ata gac tgc aac gat act gca gaa ctt gaa aca acg ttc cca 192
Glu Ser Ile Asp Cys Asn Asp Thr Ala Glu Leu Glu Thr Thr Phe Pro
50 55 60

ggg gcg tac tgg gac gag aga aac aaa aaa gcg aat ata tac aga ctg 240

Gly 65	Ala	Tyr	Trp	Asp	Glu 70	Asn	Lys	Lys	Ala 75	Ile	Tyr	Arg	Leu 80	
						aaa Lys			Arg					288
						gac Asp								336
						gat Asp 120								384
						gtc Val								432
						aca Thr								480
						gac Asp								528
						gat Asp								576
						acc Thr 200								624
						caa Gln								672
		cgc Arg												696
<210	> 11	. 6												

<211> 232

<212> PRT

<213> Toxoplasma gondii

<400> 116

Arg Gly Asn Glu Lys Thr Cys Ser Asp Ala Lys His Pro Val Tyr Ile
1 5 10 15

Lys Leu Gly Lys Gly Glu Arg Glu Ala Val Phe Lys Cys Gly Asp Gly 20 25 30

Leu Thr Thr Leu Glu Pro Ser Gln Asn Thr Asp Lys Pro Lys Phe Cys
35 40 45

Glu Ser Ile Asp Cys Asn Asp Thr Ala Glu Leu Glu Thr Thr Phe Pro
50 55 60

Gly Ala Tyr Trp Asp Glu Arg Asn Lys Lys Ala Asn Ile Tyr Arg Leu 65 70 75 80

Val Ile Pro Thr Val Ser Arg Lys Asp Thr Arg Met Tyr Tyr Lys Cys
85 90 95

Lys Gly Thr Ser Asp Ser Ala Asp Pro Cys Thr Val Leu Ile Asn Val 100 105 110

Lys Ser Thr Glu Thr Asp Asp Glu Glu Glu Glu Asp Val Gln Glu Cys
115 120 125

Thr Val Gly Thr Glu Lys Lys Val Thr Leu Ser Pro Thr Asp Thr Val 130 135 140

Lys Phe Lys Cys Asn Leu Gly Thr Val Val Gln Pro Ser Phe Ser Thr 145 150 155 160

Ala Thr Pro Lys Val Phe Asp Asp Ser Asp Gly Ser Cys Ser Ala Gln 165 170 175

Ala Ser Leu Thr Ser Leu Val Asp Ala Ser Leu Thr Glu Asp Ser Ser 180 185 190

His Gly Lys Tyr Thr Met Tyr Thr Met Asn Leu Asn Ala Arg Pro Ala 195 200 205

Glu Thr Lys Asn Leu Cys Leu Gln Cys Ser Ser Gly Lys Gln Asn Cys 210 215 220

Lys Met Arg Ile His Val Pro Ala 225 230

<210> 117

<211> 173

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(171)

<400> 117

act tgt gcg ggg gac ccc tcg gcc ttt ccg acg aag ctg ccg tcg aca 48
Thr Cys Ala Gly Asp Pro Ser Ala Phe Pro Thr Lys Leu Pro Ser Thr
1 5 10 15

cca ccc gct gct gtg ccg tct gac ggg ttg ctc gct ttg ccc tca gaa 96
Pro Pro Ala Ala Val Pro Ser Asp Gly Leu Leu Ala Leu Pro Ser Glu
20 25 30

ctt gag gcg ccg gtg gag gac ggc gac cgc gag gct ttc gtt gga gtc 144 Leu Glu Ala Pro Val Glu Asp Gly Asp Arg Glu Ala Phe Val Gly Val 35 40 45

gac ggc gcg gtc agc ggc tgg gac gag cg 173
Asp Gly Ala Val Ser Gly Trp Asp Glu
50 55

<210> 118

<211> 57

<212> PRT

<213> Toxoplasma gondii

<400> 118

Thr Cys Ala Gly Asp Pro Ser Ala Phe Pro Thr Lys Leu Pro Ser Thr 1 5 10 15

Pro Pro Ala Ala Val Pro Ser Asp Gly Leu Leu Ala Leu Pro Ser Glu 20 25 30

Leu Glu Ala Pro Val Glu Asp Gly Asp Arg Glu Ala Phe Val Gly Val 35 40 45

Asp Gly Ala Val Ser Gly Trp Asp Glu 50 55

<210> 119 <211> 369

104

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(369)

<400> 119

cgc tct gtg ttt cag gtc gcg agc gac gcg aga aac gcc cga cag gcg 48
Arg Ser Val Phe Gln Val Ala Ser Asp Ala Arg Asn Ala Arg Gln Ala
1 5 10 15

acc tcg ggc gtg ccg cgg cag agg gga aag aag gcc gtc acg gcg cga 96
Thr Ser Gly Val Pro Arg Gln Arg Gly Lys Lys Ala Val Thr Ala Arg
20 25 30

gtc tct ttc ggc gct cta gag gag aga gac agt tcg agt tcg gac gtt 144
Val Ser Phe Gly Ala Leu Glu Glu Arg Asp Ser Ser Ser Ser Asp Val
35 40 45

ccc gag gaa agg gat aaa gac gcc gaa aac ggc tct gcg cct cgc atc 192 Pro Glu Glu Arg Asp Lys Asp Ala Glu Asn Gly Ser Ala Pro Arg Ile 50 55 60

ttc gcg tct tct tcc ctg acg cgg ctt tcg cct cct tct ctc tct ccg 240
Phe Ala Ser Ser Ser Leu Thr Arg Leu Ser Pro Pro Ser Leu Ser Pro
65 70 75. 80

ctc tca agt tcg ggg cca tct tca ccg tct tct tcc gtt tcg cgg ttt 288
Leu Ser Ser Ser Gly Pro Ser Ser Pro Ser Ser Ser Val Ser Arg Phe
85 90 95

acc gac tcc ctg ccg cag tcg acg gct tcg tct cgt ctc tcc tct gct 336

Thr Asp Ser Leu Pro Gln Ser Thr Ala Ser Ser Arg Leu Ser Ser Ala

100 105 110

tat tog oft gag tog ogt ogg oct oft gag ocg
Tyr Ser Leu Glu Ser Arg Arg Pro Leu Glu Pro
115 120

<210> 120

<211> 123

<212> PRT

<213> Toxoplasma gondii

<400> 120

Arg Ser Val Phe Gln Val Ala Ser Asp Ala Arg Asn Ala Arg Gln Ala

1 5 10 15

Thr Ser Gly Val Pro Arg Gln Arg Gly Lys Lys Ala Val Thr Ala Arg
20 25 30

Val Ser Phe Gly Ala Leu Glu Glu Arg Asp Ser Ser Ser Ser Asp Val 35 40 45

Pro Glu Glu Arg Asp Lys Asp Ala Glu Asn Gly Ser Ala Pro Arg Ile 50 55 60

Phe Ala Ser Ser Ser Leu Thr Arg Leu Ser Pro Pro Ser Leu Ser Pro 65 70 75 80

Leu Ser Ser Ser Gly Pro Ser Ser Fro Ser Ser Ser Val Ser Arg Phe
85 90 95

Thr Asp Ser Leu Pro Gln Ser Thr Ala Ser Ser Arg Leu Ser Ser Ala 100 105 110

Tyr Ser Leu Glu Ser Arg Arg Pro Leu Glu Pro 115 120

<210> 121

<211> 566

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(183)

<400> 121

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Arg Arg Trp Met Thr Gly Ala Asn Tyr Glu Gly His Gln Gly Gln Tyr
1 5 10 15

ttg aac tac tgc acc att tct cac ttc ttg tgt tgc cct aat ggg atc 96 Leu Asn Tyr Cys Thr Ile Ser His Phe Leu Cys Cys Pro Asn Gly Ile 20 25 30

tgt cgt ttt caa tgg gac aat cag ccc agt ctc gat agg gag gac tca 144 Cys Arg Phe Gln Trp Asp Asn Gln Pro Ser Leu Asp Arg Glu Asp Ser 35 40 45

atc tgg tgc tct gaa tcg att tct cgt ttt cgc ctg agc taagataact 193 Ile Trp Cys Ser Glu Ser Ile Ser Arg Phe Arg Leu Ser

50 55 60

actgraagaca tttgtagacg ctttctacaa acccacgtgg caaaatctta cggaaggaca 253
aatgcctctt tcaacactct tctttcatcg ctgcttgtta cactcctgag aggccccaag 313
agccacggtg ccactttgct tccccagccg ctactgtgca aattcttat agaagagcac 373
aaatgttccc cgaagaagca gcagcacct ttgaggagcc tgaagagcga ccctacgaat 433
cacagcgttc agaaatagcc tactgtagta ttaaggagac taccaaagtg aaaatcgtga 493
tatgtctaca ggtggtatgc aagtgttggt tttccagata tacgctgcaa ctaaaacacc 553
aaaatgatag aat 566

<210> 122

<211> 61

<212> PRT

<213> Toxoplasma gondii

<400> 122

Arg Arg Trp Met Thr Gly Ala Asn Tyr Glu Gly His Gln Gly Gln Tyr

1 5 10 15

Leu Asn Tyr Cys Thr Ile Ser His Phe Leu Cys Cys Pro Asn Gly Ile 20 25 30

Cys Arg Phe Gln Trp Asp Asn Gln Pro Ser Leu Asp Arg Glu Asp Ser 35 40 45

Ile Trp Cys Ser Glu Ser Ile Ser Arg Phe Arg Leu Ser 50 55 60

<210> 123

<211> 616

<212> DNA

<213> Toxoplasma gondii

<220> .

<221> CDS

<222> (1)..(615)

<400> 123

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wo	99/3	2633													PCT/	US98/27137
1				5					10					15		
cag Gln					cga Arg							-	•	-		96
tcc	tct	tgt	tcc	tcc	agc	aac	tca	cag	aac	cct	ccg	caa	gat	tcc	tcg	144

cac gtc tgc tgt ccc tcc tcc tct gcc ttc tcc cag ccg cgc tct tct 192
His Val Cys Cys Pro Ser Ser Ser Ala Phe Ser Gln Pro Arg Ser Ser
50 55 60

Ser Ser Cys Ser Ser Ser Asn Ser Gln Asn Pro Pro Gln Asp Ser Ser

40

35

ctg tcc tca tcc tca ccc tct tcg tct gcc gcg tta cca tcg ggg tct 240 Leu Ser Ser Ser Ser Pro Ser Ser Ser Ala Ala Leu Pro Ser Gly Ser 65 70 75 80

tct ccc tcg gct gcg tct tcg tct cat gca ctt ggg gtg gtg gac tcg 288 Ser Pro Ser Ala Ala Ser Ser Ser His Ala Leu Gly Val Val Asp Ser 85 90 95

gac cgg att tct gcg gag gag gcg tcc ctc gag gag gcc cgg cgg 336 Asp Arg Ile Ser Ala Glu Glu Ala Ala Ser Leu Glu Glu Ala Arg Arg 100 105 110

ctg cag aga cag ttc gag gcg gaa atg gtg ggc att cga ccg cca gac 384 Leu Gln Arg Gln Phe Glu Ala Glu Met Val Gly Ile Arg Pro Pro Asp 115 120 125

gac acc tac gag gaa acg ctg att tct gag gac atc cat cct tcc cac 432
Asp Thr Tyr Glu Glu Thr Leu Ile Ser Glu Asp Ile His Pro Ser His
130 135 140

cga gcc tgg tgg gaa aga cct agc gcc tcg ccg att cgt ctg tcg cgc 480 Arg Ala Trp Trp Glu Arg Pro Ser Ala Ser Pro Ile Arg Leu Ser Arg 145 150 155 160

gcg gcg tcg atg aga agt gac ggt cgc aga ggt caa cag ccc ccg agt 528
Ala Ala Ser Met Arg Ser Asp Gly Arg Arg Gly Gln Gln Pro Pro Ser
165 170 175

cga cag tct cct cag gac ggg gag gaa gac gac gcc gct ctc gcc aga 576 Arg Gln Ser Pro Gln Asp Gly Glu Glu Asp Asp Ala Ala Leu Ala Arg 180 185 190

cga ctt cag gaa gaa tac agc cga cat cga gag gtc g 616 Arg Leu Gln Glu Glu Tyr Ser Arg His Arg Glu Val

195 200 205

<210> 124

<211> 205

<212> PRT

<213> Toxoplasma gondii .

<400> 124

His Glu Arg Arg Val Ala Glu Glu Lys Ala Arg Glu Glu Arg Glu Arg 1 5 10 15

Gln Ala Ala Ser Gln Arg Asn Gly Ser Thr Glu Pro Ala Val Ala Pro 20 25 30

Ser Ser Cys Ser Ser Ser Asn Ser Gln Asn Pro Pro Gln Asp Ser Ser 35 40 45

His Val Cys Cys Pro Ser Ser Ser Ala Phe Ser Gln Pro Arg \mathfrak{Ser} \mathfrak{Ser} 50 55 60

Leu Ser Ser Ser Ser Pro Ser Ser Ser Ala Ala Leu Pro Ser Gly Ser 65 70 75 80

Ser Pro Ser Ala Ala Ser Ser Ser His Ala Leu Gly Val Val Art Ger 85 90 96

Asp Arg Ile Ser Ala Glu Glu Ala Ala Ser Leu Glu Glu Ala Arg Arg 100 105 110

Leu Gln Arg Gln Phe Glu Ala Glu Met Val Gly Ile Arg Pro iro Aug 115 120 125

Arg Ala Trp Trp Glu Arg Pro Ser Ala Ser Pro Ile Arg Leu (**: Arg 145 - 150 - 155 - 1**

Ala Ala Ser Met Arg Ser Asp Gly Arg Gly Gln Gln Fro Fr 1007

Arg Gln Ser Pro Gln Asp Gly Glu Glu Asp Asp Ala Ala Leu Ala Ari 180 185 190

Arg Leu Gln Glu Glu Glu Tyr Ser Arg His Arg Glu Val 195 200 205

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WO 99/32633 PCT/US98/27137 Pro Glu Pro Leu Pro Glu Ala Pro Glu Glu Ser Glu Gln Ala Pro Glu 150 cca ctg ccc gag gca ccc gaa gaa tcc gag cag gct cct gag cca ctg 528 Pro Leu Pro Glu Ala Pro Glu Glu Ser Glu Gln Ala Pro Glu Pro Leu 165 170 ccc gag gca ccc gaa gaa ttt gac cag gct cct atg cca ctg ccc gcg 576 Pro Glu Ala Pro Glu Glu Phe Asp Gln Ala Pro Met Pro Leu Pro Ala 180 gcc ccc gaa gac ttt gac cag cct gct atg cca ctg ccc ccg gcc ccc Ala Pro Glu Asp Phe Asp Gln Pro Ala Met Pro Leu Pro Pro Ala Pro 195 200 gaa gac ttt gac cag gct ccc atg cca ctg ccg cag gca ccc gaa gaa 672 Glu Asp Phe Asp Gln Ala Pro Met Pro Leu Pro Gln Ala Pro Glu Glu 210 215 ctc gag cag gct ccc gct tcc acc ccg agg agg cgg agc agg agg tgc 720 Leu Glu Gln Ala Pro Ala Ser Thr Pro Arg Arg Arg Ser Arg Arg Cys 225 230 235 ctg aga gaa aaa ctg acg cag gaa gtg aac ctg aga agg atc 762 Leu Arg Glu Lys Leu Thr Gln Glu Val Asn Leu Arg Arg Ile 245 250 <210> 126 <211> 254 <212> PRT <213> Toxoplasma gondii Arg Asp Gln Ala Pro Lys Pro Val Pro Glu Ala Ala Asp Glu Phe Asp 5 Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala 20 25 Pro Glu Pro Leu Arg Glu Ala Ala Glu Glu Phe Asp Gln Ala Pro Met 35 40 Pro Val Pro Glu Ala Pro Glu Asp Phe Asp Gln Ile Pro Lys Pro Val

70

Pro Glu Ala Pro Glu Glu Phe Asp Gln Ala Pro Met Pro Val Pro Glu

75

65

Ala Pro Glu Asp Phe Asp Gln Ile Pro Lys Pro Val Pro Glu Ala Pro 85 90 95

Glu Glu Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Glu 100 105 110

Ser Glu Gln Ala Pro Glu Pro Leu Pro Glu Ala Pro Glu Glu Ser Glu 115 120 125

Gln Ala Pro Glu Pro Leu Pro Glu Ala Pro Glu Glu Ser Glu Gln Ala 130 135 140

Pro Glu Pro Leu Pro Glu Ala Pro Glu Glu Ser Glu Gln Ala fro Glu 145 150 155 160

Pro Leu Pro Glu Ala Pro Glu Glu Ser Glu Gln Ala Pro Glu Pro Leu 165 170 175

Pro Glu Ala Pro Glu Glu Phe Asp Gln Ala Pro Met Pro Leu Frc Ala 180 185 190

Ala Pro Glu Asp Phe Asp Gln Pro Ala Met Pro Leu Pro Pro Ala Pro 195 200 205

Glu Asp Phe Asp Gln Ala Pro Met Pro Leu Pro Gln Ala Pro Glu 71u 210 215 220

Leu Glu Gln Ala Pro Ala Ser Thr Pro Arg Arg Arg Ser Arg Ar : Cys 225 230 235 246

Leu Arg Glu Lys Leu Thr Gln Glu Val Asn Leu Arg Arg Ile 245 250

<210> 127

<211> 236

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(234)

<400> 127

cgc gga gag ggg gag act gag aga ggg cag aat gag gag act cac gca 48
Arg Gly Glu Gly Glu Thr Glu Arg Gly Gln Asn Glu Glu Thr His Ala
1 5. 10

acg aac aag too toa ggc gtc gcc agt ttg gag gca cca gcg tcg ttc Thr Asn Lys Ser Ser Gly Val Ala Ser Leu Glu Ala Pro Ala Ser Phe 20 25 144 Ala Gln Glu Gly Asp Gly Gly Arg Arg Glu Glu Ala Ser Gln Ala Lys 40 atg ggg acg tct ccc ccg tcg aat cag gtg atc aac gtt gta gac gaa 192 Met Gly Thr Ser Pro Pro Ser Asn Gln Val Ile Asn Val Val Asp Glu 50 55 . 60 gac gag gag gac gac gag gaa gca gag gcg cta gag gct ccc gg 236 Asp Glu Glu Asp Asp Glu Glu Ala Glu Ala Leu Glu Ala Pro 65 70 <210> 128 <211> 78 <212> PRT <213> Toxoplasma gondii <400> 128 Arg Gly Glu Gly Glu Thr Glu Arg Gly Gln Asn Glu Glu Thr His Ala 1 5 Thr Asn Lys Ser Ser Gly Val Ala Ser Leu Glu Ala Pro Ala Ser Phe 20 25 30 Ala Gln Glu Gly Asp Gly Gly Arg Arg Glu Glu Ala Ser Gln Ala Lys 35 40 Met Gly Thr Ser Pro Pro Ser Asn Gln Val Ile Asn Val Val Asp Glu 55 Asp Glu Glu Asp Asp Glu Glu Ala Glu Ala Leu Glu Ala Pro 70 <210> 129 <211> 569 <212> DNA <213> Toxoplasma gondii <220> <221> CDS <222> (1)..(567)

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aca ctt gtt aat gca agc tgg ctt ggt gcc tct gca ggg gag act ato Thr Leu Val Asn Ala Ser Trp Leu Gly Ala Ser Ala Gly Glu Thr Ile 20 25 30	c 96
gct gat tca agg gct tta agg cgt gac cta tca ttc cca ctg tct agt Ala Asp Ser Arg Ala Leu Arg Arg Asp Leu Ser Phe Pro Leu Ser Ser 35 40 45	144
cgt caa ctg cga gaa cgt ggc ctt gct tct caa gat tcc tca ctt tca Arg Gln Leu Arg Glu Arg Gly Leu Ala Ser Gln Asp Ser Ser Leu Ser 50 55 60	192
agc act cca aaa ttg tcc ctg caa cac gac cac ttt gca aag act ctg Ser Thr Pro Lys Leu Ser Leu Gln His Asp His Phe Ala Lys Thr Leu 65 70 75 80	240
gta aaa cga aga gcg ctg tct gca acg aac tcc aca gaa cgc agc ggc Val Lys Arg Arg Ala Leu Ser Ala Thr Asn Ser Thr Glu Arg Ser Gly 85 90 95	288
aaa cca gtt cgt tgc ttt act gaa acc agc gtg agg tta ggt gca cct Lys Pro Val Arg Cys Phe Thr Glu Thr Ser Val Arg Leu Gly Ala Pro 100 105 110	336
act caa ccg gta atg gag gaa atg cct ttg gga gaa gga gag gta aat Thr Gln Pro Val Met Glu Glu Met Pro Leu Gly Glu Gly Glu Val Asn 115 120 125	384
ctg gtc tcc gaa cac gac gat tat gca gaa tcc acc agt cat ctg gat Leu Val Ser Glu His Asp Asp Tyr Ala Glu Ser Thr Ser His Leu Asp 130 135	432
acg gtg aat ggg aga gaa aga aga gag gaa agg cat tac gcg gag acg Thr Val Asn Gly Arg Glu Arg Arg Glu Glu Arg His Tyr Ala Glu Thr 150 155 160	480
gag gcg aca gac gaa ttc aaa tcc gca atg cac cac gtg acg tcg ccc Glu Ala Thr Asp Glu Phe Lys Ser Ala Met His His Val Thr Ser Pro 165 170 175	528
gga ggg gta ccc gca acg aaa aag gtg gtg tgg aag atc cg Gly Gly Val Pro Ala Thr Lys Lys Val Val Trp Lys Ile	569

180 185

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<211> 189

<212> PRT

<213> Toxoplasma gondii

<400> 130

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1 5 10 15

Thr Leu Val Asn Ala Ser Trp Leu Gly Ala Ser Ala Gly Glu Thr Ile 20 25 30

Ala Asp Ser Arg Ala Leu Arg Arg Asp Leu Ser Phe Pro Leu Ser Ser 35 40 45

Arg Gln Leu Arg Glu Arg Gly Leu Ala Ser Gln Asp Ser Ser Leu Ser 50 55 60

Ser Thr Pro Lys Leu Ser Leu Gln His Asp His Phe Ala Lys Thr Leu 65 70 75 80

Val Lys Arg Arg Ala Leu Ser Ala Thr Asn Ser Thr Glu Arg Ser Gly
85 90 95

Lys Pro Val Arg Cys Phe Thr Glu Thr Ser Val Arg Leu Gly Ala Pro 100 105 110

Thr Gln Pro Val Met Glu Glu Met Pro Leu Gly Glu Gly Glu Val Asn 115 120 125

Leu Val Ser Glu His Asp Asp Tyr Ala Glu Ser Thr Ser His Leu Asp 130 135 140

Thr Val Asn Gly Arg Glu Arg Glu Glu Arg His Tyr Ala Glu Thr 145 150 155 160

Glu Ala Thr Asp Glu Phe Lys Ser Ala Met His His Val Thr Ser Pro 165 170 175

Gly Gly Val Pro Ala Thr Lys Lys Val Val Trp Lys Ile 180 185

<210> 131

<211> 232

<212> DNA

<213> Toxoplasma gondii

<400> 131

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gacacagece caaacteeeg acagegtgtt getetgtegg geaggeagge caagetggea 180

ageogetage atgecacgtg etgtactget ggeoegaaac tacagtgege ac 232

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<211> 276

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<220>

<221> CDS

<222> (1)..(276)

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ccc gga att ccg gct ccg ggt cgc aaa gcg atc cat ttg ata aaa gac
Pro Gly Ile Pro Ala Pro Gly Arg Lys Ala Ile His Leu Ile Lys Asp
1 5 10 15

tgc gtt ttc tgc ctt ggg gaa ctc ttc ttg aat ggc acg aga ggc cac 96 Cys Val Phe Cys Leu Gly Glu Leu Phe Leu Asn Gly Thr Arg Gly His 20 25 30

gcg ccc aga ata cag gca gcc tct ccg aag tca ctc acc ttg tac gat 192
Ala Pro Arg Ile Gln Ala Ala Ser Pro Lys Ser Leu Thr Leu Tyr Asp
50 55 60

ctt gtg cac agt gat gta ggg cgc atg cag aac gac gcc tcc aac atg 240 Leu Val His Ser Asp Val Gly Arg Met Gln Asn Asp Ala Ser Asn Met 65 70 75 80

aat att ctc ctc ggc caa ggc cgc cgc caa gta gcg
Asn Ile Leu Leu Gly Gln Gly Arg Arg Gln Val Ala

85

<210> 133

<211> 92

<212> PRT

<213> Toxoplasma gondii

<400> 133

Pro Gly Ile Pro Ala Pro Gly Arg Lys Ala Ile His Leu Ile Lys Asp 1 5 10 15

Cys Val Phe Cys Leu Gly Glu Leu Phe Leu Asn Gly Thr Arg Gly His 20 25 30

Arg Gln Arg Glu Arg Glu Gly Lys Pro Lys Lys Gln Thr Gly Ser Glu 35 40 45

Ala Pro Arg Ile Gln Ala Ala Ser Pro Lys Ser Leu Thr Leu Tyr Asp 50 55 60

Leu Val His Ser Asp Val Gly Arg Met Gln Asn Asp Ala Ser Asn Met 65 70 75 80

Asn Ile Leu Leu Gly Gln Gly Arg Arg Gln Val Ala 85 90

<210> 134

<211> 309

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(309)

<400> 134

cgc gga cac act gga gag agg tgg tcg gac agg gag gga gaa tcc gag 48
Arg Gly His Thr Gly Glu Arg Trp Ser Asp Arg Glu Gly Glu Ser Glu
1 5 10 15

atg tgc agt gga gga caa atg gaa aag aga gag agc cga cgc gtt tct 96
Met Cys Ser Gly Gly Gln Met Glu Lys Arg Glu Ser Arg Arg Val Ser
20 25 30

ttt gcg gat gaa gag atg cgg aat ccg aca gaa aac ctg aag gta gat 144 Phe Ala Asp Glu Glu Met Arg Asn Pro Thr Glu Asn Leu Lys Val Asp 35 40 45

gcc aac tgt gtg ctc gaa ggt ctg tcc acc tca gtg tgt gcg agg cgg 192

75

Ala Asn Cys Val Leu Glu Gly Leu Ser Thr Ser Val Cys Ala Arg Arg

ctg aag agg caa aag cga act gca ggt cag tct ggc ttc ctc gca ata Leu Lys Arg Gln Lys Arg Thr Ala Gly Gln Ser Gly Phe Leu Ala Ile

cga aac gtc caa ggc acc gcg acc gcc cta aaa cac cct gat tcc aca Arg Asn Val Gln Gly Thr Ala Thr Ala Leu Lys His Pro Asp Ser Thr 288 90

gga cga cgg tct tgg gat ccg Gly Arg Arg Ser Trp Asp Pro 309 100

<210> 135

<211> 103

<212> PRT

<213> Toxoplasma gondii

<400> 135

Arg Gly His Thr Gly Glu Arg Trp Ser Asp Arg Glu Gly Glu Ser Glu

Met Cys Ser Gly Gly Gln Met Glu Lys Arg Glu Ser Arg Arg Val Ser 25

Phe Ala Asp Glu Glu Met Arg Asn Pro Thr Glu Asn Leu Lys Val Asp 40 45

Ala Asn Cys Val Leu Glu Gly Leu Ser Thr Ser Val Cys Ala Arg Arg

Leu Lys Arg Gln Lys Arg Thr Ala Gly Gln Ser Gly Phe Leu Ala Ile 75

Arg Asn Val Gln Gly Thr Ala Thr Ala Leu Lys His Pro Asp Ser Thr 90

Gly Arg Arg Ser Trp Asp Pro 100

<210> 136

<211> 534

<212> DNA

<213> Toxoplasma gondii

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165 . 170

gtg ctg Val Leu 534

<210> 137

<211> 178

<212> PRT

<213> Toxoplasma gondii

<400> 137

Arg Ile Glu Ala Glu Ile Ala Arg Gln Lys Glu Arg Glu Ala Lys Leu 1 5 10 15

Arg Arg Arg Leu Ala Ala Val Val Ala Ser Met Leu Val Ala Ala Ser 20 25 30

Leu Tyr Gly Leu Asn Ser Phe Leu His Gly Ser Asp Lys Glu Ile Ser 35 40 45

Ser Met Pro Ser Ser Ile Asp Lys Lys Pro Asp Ser Pro Phe Ala Ala 50 55 60 .

Gln Leu Gly Thr Ser Leu Glu Ser Glu Ile Gly Ile Pro Glu Glu Lys
65 70 75 80

Ala Ile Pro Glu Ala Ala Asp Ile Ser Ser Phe Ile Glu Asn Leu Ser 85 90 95

Ala Thr Val Ala Gly Asn Ser Val Gln Ala Gln Ser Ile Gly Phe Val

Leu Thr Val Val Leu Gly Leu Val Ala Phe Ser Leu Lys Ala Ala 115 120 125

Arg Arg Ser Ser Pro Arg Glu Glu Gln Ala Phe Ser Leu Pro Ala His 130 135 140

Pro Pro Arg Glu Glu Lys Ser Lys Tyr Leu Leu Lys Pro Pro Gln Gln 145 150 155 160

Pro Lys Pro Arg Arg Leu Lys Arg Gln Leu Arg Lys Tyr Arg Gln Arg 165 170 175

Val Leu

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<211> 423
<212> DNA
<213> Toxoplasma gondii
<220>
\langle 223 \rangle At locations 6, 23 and 34, N = unknown
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gtccggtgga ggatgttgtt gagcccccgt cgggagtgga agacctgccg cagccagaga 120
cagaggegea agtacegace aagggtgttg accatgeege gtegggaggg gaggaeuteg 180
tggagccaga ggcagagccg cagggactgg tggctggcgc tggtgaggcc gcatcgqqaq 240
gggaggacct gctagagcca ggggcagcgc cgcagggtcc ggtgaaggat attmitquiq 300
cggcgtcggg agaggaagaa ctgctggagc cagaggcaaa gccgcagggt tmartaluar 360
atgttgatga ggcagcgtcg ggaggggagg acctgctaga gccagaggca quanchquaq 420
tcc
                                                                    423
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<212> DNA
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<220>
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<222> (1)..(327)
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ego tet caa tea aca aag eea eee geg eet tea gae gta gag \sigma a = a \cup a
                                                                    48
Arg Ser Gln Ser Thr Lys Pro Pro Ala Pro Ser Asp Val Glu App Thr
ggo tot tot gad aad dog ggt gad aat gtg ada gag gad ada adt gag
Gly Ser Ser Asp Asn Pro Gly Asp Asn Val Thr Glu Asp Thr Thr Glu
             20
                                                       30
agt cca tca cag ggc acc gac ggt tca gca tcc gga ccc ggg tcq act
Ser Pro Ser Gln Gly Thr Asp Gly Ser Ala Ser Gly Pro Gly Ser Thr
         35
                              40
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121

cat ccg gaa aac gac gcg ggg gaa cat gag gat ggc gcg tca ctg ggg His Pro Glu Asn Asp Ala Gly Glu His Glu Asp Gly Ala Ser Leu Gly 50 55	192
Caa gac cag caa gag cgc atg gat aaa tct tcc cta ggc aaa gaa aca Gln Asp Gln Gln Glu Arg Met Asp Lys Ser Ser Leu Gly Lys Glu Thr 70 75 80	240
ccc atg ctc gat cag gga aat tcg tca cca gca aca acg ggg tcc ggt Pro Met Leu Asp Gln Gly Asn Ser Ser Pro Ala Thr Thr Gly Ser Gly 85 90 95	288
gcc cat gaa aaa aac gag agc gtg tca gga gtt cca gcg Ala His Glu Lys Asn Glu Ser Val Ser Gly Val Pro Ala 100 105	327
<pre><210> 140 <211> 109 <212> PRT <213> Toxoplasma gondii <400> 140 Arg Ser Gln Ser Thr Lys Pro Pro Ala Pro Ser Asp Val Glu Asp Thr 1</pre>	
Ser Pro Ser Gln Gly Thr Asp Gly Ser Ala Ser Gly Pro Gly Ser Thr 35 40 45	
His Pro Glu Asn Asp Ala Gly Glu His Glu Asp Gly Ala Ser Leu Gly 50 60	
Gln Asp Gln Glu Arg Met Asp Lys Ser Ser Leu Gly Lys Glu Thr 65 70 75 80	
Pro Met Leu Asp Gln Gly Asn Ser Ser Pro Ala Thr Thr Gly Ser Gly 85 90 95	
Ala His Glu Lys Asn Glu Ser Val Ser Gly Val Pro Ala 100 105	
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122

PCT/US98/27137

WO 99/32633 <212> DNA <213> Toxoplasma gondii <220> <221> CDS <222> (1) .. (444) <400> 141 ceg geg ega aet gge gae geg eag eet gag gge aga gag ggg eac age Pro Ala Arg Thr Gly Asp Ala Gln Pro Glu Gly Arg Glu Gly His Ser 1 5 cca ctg gaa gac gaa ggg aga gat gcg ttt gga aga cgc gct gcg gaa Pro Leu Glu Asp Glu Gly Arg Asp Ala Phe Gly Arg Arg Ala Ala Glu 25 gac gag aga aac aga gga aat ccg aat gcg gct ggc gag act tcc caa Asp Glu Arg Asn Arg Gly Asn Pro Asn Ala Ala Gly Glu Thr Ser Gln 35 40 gac gag gca gag aac gcg caa gcg tcc ctg cgg ttc gct gcg aga gag 192 Asp Glu Ala Glu Asn Ala Gln Ala Ser Leu Arg Phe Ala Ala Arg Glu 55 aaa cct ctc gaa gtc ctc aga ttc cga gaa gac act gca gac act ctg Lys Pro Leu Glu Val Leu Arg Phe Arg Glu Asp Thr Ala Asp Thr Leu 65 70 75 acg tat gca gac tat cca aac agc gtg gag ttc aca ccc gca gac atg Thr Tyr Ala Asp Tyr Pro Asn Ser Val Glu Phe Thr Pro Ala Asp Met 85 95 ccg aat gcg aag gac cag acg cct ctg cat gca aag tac aat cac ttt 336 Pro Asn Ala Lys Asp Gln Thr Pro Leu His Ala Lys Tyr Asn His Phe-100 105

tgc gcc tac tca tgc tgg ctg acc tcg cgc ttc aac cca gac aac cca 384 Cys Ala Tyr Ser Cys Trp Leu Thr Ser Arg Phe Asn Pro Asp Asn Pro

> 115 120 125

ame age cae tgt gga aaa gga aaa aae gag aaa ege ega tte gae gae 432 Asn Ser His Cys Gly Lys Gly Lys Asn Glu Lys Arg Arg Phe Asp Asp 135 140

gac tac gat ccg 444 Asp Tyr Asp Pro 145

<210> 142

<211> 148

<212> PRT

<213> Toxoplasma gondii

<400> 142

Pro Ala Arg Thr Gly Asp Ala Gln Pro Glu Gly Arg Glu Gly His Ser 1 5 10 15

Pro Leu Glu Asp Glu Gly Arg Asp Ala Phe Gly Arg Arg Ala Ala Glu 20 25 30

Asp Glu Arg Asn Arg Gly Asn Pro Asn Ala Ala Gly Glu Thr Ser Gln 35 40 45

Asp Glu Ala Glu Asn Ala Gln Ala Ser Leu Arg Phe Ala Ala Arg Glu 50 55 60

Lys Pro Leu Glu Val Leu Arg Phe Arg Glu Asp Thr Ala Asp Thr Leu 65 70 75 80

Thr Tyr Ala Asp Tyr Pro Asn Ser Val Glu Phe Thr Pro Ala Asp Met 85 90 95

Pro Asn Ala Lys Asp Gln Thr Pro Leu His Ala Lys Tyr Asn His Phe 100 105 110

Cys Ala Tyr Ser Cys Trp Leu Thr Ser Arg Phe Asn Pro Asp Asn Pro 115 120 125

Asn Ser His Cys Gly Lys Gly Lys Asn Glu Lys Arg Arg Phe Asp Asp 130 135 140

Asp Tyr Asp Pro 145

<210> 143

<211> 928

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(57)

<400> 143

48

cgg gat ccc gag cgg gac ctc ccg gtg tcc tcg act gct cat aca cca

Arg Asp Pro Glu Arg Asp Leu Pro Val Ser Ser Thr Ala His Thr Pro 1 5 15 gag gag gat tgattcaccg cagaacttaa cccgtgggac gcagcctcca 97 Glu Glu Asp caqaqtctqt ggcgqacqaa ggaaatgcag gaacagaqag acctgqaccq aaaaatggca 157 tggacgatgg gtgtccacgg gagaagacgg atactcgtgg aaagccgggg gaaagcgacg 217 qaqqqaaatg cqcqacaagc tggaaaagcg agctcacaac gacqaaacac qcactqtqca 277 tecgaacgae aatgaeetgt eettagtaga egagaggggg taggeaacaa tteeteagaa 337 gtccaccage gaceggacag egacegegge ageaegttga gggaggtett actageggee 397 gagactcagg acaacaggag ccctctaccg ccatgcgaca cacgcagaac aacgctttag 457 attaaggtcg aaaaaggaaa cctcaacgca gaacgagtca cttctttccc acaaaagtgc 517 tgtggaaaaa cagcgcatgc ggggctgggt gactcgaaaa tctgggaacg cgtctggcag 577 gcatcctgcc cgaacccgat accagagaaa cggaacccgt actggctgga attcaacagt 637 acggacaaaa aacccaccgt gtaaagtgga caaaagccga caatggaaca actacttggg 697 agaaagcaaa tgctgcgttc accaggccag tgtcacgccg cgtcgtaaag aaggcgqctc 757 agegttgeeg gtgtgeetgg egttgtegga eggetteegt gtgtaeecaa eagaaaaget 817 tttacactct tactattttc atttggccac gatttctttt ttgcctatct actgtaccta 877 gccacgtcgc cattctaagg aagttgtccc gttgcaacgc agaacgcgga g 928

<210> 144

<211> 19

<212> PRT

<213> Toxoplasma gondii

<400> 144

Arg Asp Pro Glu Arg Asp Leu Pro Val Ser Ser Thr Ala His Thr Pro 1 5 10 15

Glu Glu Asp

WO 99/32633	PCT/US98/27137
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gcaccttgtt ctctctcttc gcc	23
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cgaggagacg gtgggagc	18
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tgcccaagat gccgatctct g	21
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agctgo	gcaga aataccaaag ctc		23
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<210>	153		
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	Primer		
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gacge	igaga agaaagaaga gee		
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	Primer		
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.010-	156		
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<220>			
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-2237	Drimer	- 4	

wo	99/32633	PCT/US98/27
<400>	156	
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55		21
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WO 99/32633	PCT/US98/27137
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gautecated egracuated g	21
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Primer	
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caagacacag ggaaacgttg g	21

wo s	99/32633	PCT/US98/27137
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	Artificial Sequence	
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	Description of Artificial Sequence: Synthetic	
	Primer	
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WO 99/32633	PCT/US98/2713
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4210. 162	
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Primer	
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Primer	
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gaagatgctt gtcttgttcg gttc	24
	2.
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		gta Val														96
		ccg Pro 35														144
		cgg Arg														192
		cga Arg														240
aga	cgg	tct	gcc	gtc	gac	gat	tcg	ata	ccg	gct	aac	ccc	atc	gct	ttg	288

PCT/US98/27137

WO 99/32633

Arg Arg Ser Ala Val Asp Asp Ser Ile Pro Ala Asn Pro Ile Ala Leu 85 90 95

aacgcgtggc gccctgcgat ccg

311

<210> 266

<211> 96

<212> PRT

<213> Toxoplasma gondii

<400> 266

Arg Asp Pro Ala Ala Pro Asn Ser Thr Gln Ala Val Ala Ala Ala Arg 1 5 10 15

Thr Val Val Met Lys Thr Asp Ala Glu Val Ser Gly Asp Asn Leu 20 25 30

Ser Gln Pro Gly Arg Arg Pro Pro Ser Pro Lys Pro Gln Thr Thr Lys
35 40 45

Phe Pro Arg Arg Glu Ser Pro Asp Arg Gly Thr Arg Arg Arg Thr
50 55 60 .

Glu Ser Arg Gly Ala Val Ser Arg Val Trp Pro Gly Glu Asn Gln Arg
65 70 75 80

Arg Arg Ser Ala Val Asp Asp Ser Ile Pro Ala Asn Pro Ile Ala Leu 85 90 95

<210> 267

<211> 303

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(303)

<400> 267

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Asp Glu Ala Leu Pro Leu Phe Gly Ala Asn Asp Gly Thr Ser Val Arg
1 5 10 15

ctc tcc ctc gac cgc agc gtc ctg ctt gtt ctc gaa ccc gca gag ccc 96 Leu Ser Leu Asp Arg Ser Val Leu Leu Val Leu Glu Pro Ala Glu Pro 20 25 30

														ctt		144
Leu	Leu	Ser 35	Ser	Trp	Pro	His	Pro 40	Gly	Arg	Arg	Asp	Thr 45	Phe	Leu	Glu	
														cgc		192
Gly		Gly	Ala	Gly	Ile		Ser	Pro	Ser	Ser		Pro	Ser	Arg	Ala	
	50					55					60					
gcc	gac	cat	tac	acg	aga	ctc	tcc	acg	att	cgg	tct	ctt	gcc	agg	gat	240
Ala	Asp	His	Tyr	Thr	Arg	Leu	Ser	Thr	Ile	Arg	Ser	Leu	Ala	Arg	Asp	
65					70					75					80	
aaa	gag	atc	gac	tcc	gag	cta	aca	aaa	aas	cca	C 3 C	<i>α</i>	242	gaa	201	200
														Glu		288
•			•	85					90			014	9	95	50.	
_	_	gtg	-	_												303
Val	Arg	Val	_	Pro												
			100													
<210	> 26	8														
	> 10	_														
<212> PRT																

<213> Toxoplasma gondii

<400> 268

Asp Glu Ala Leu Pro Leu Phe Gly Ala Asn Asp Gly Thr Ser Val Arg 10

Leu Ser Leu Asp Arg Ser Val Leu Leu Val Leu Glu Pro Ala Glu Pro 25

Leu Leu Ser Ser Trp Pro His Pro Gly Arg Arg Asp Thr Phe Leu Glu 40

Gly Asp Gly Ala Gly Ile Pro Ser Pro Ser Ser Arg Pro Ser Arg Ala 50 55

Ala Asp His Tyr Thr Arg Leu Ser Thr Ile Arg Ser Leu Ala Arg Asp 65 70 75

Gly Glu Val Asp Ser Glu Leu Ala Gly Gly Pro Gln Glu Arg Glu Ser 90 85

Val Arg Val Asp Pro 100

160

<210> 269 <211> 236 <212> DNA <213> Toxoplasma gondii <220> <221> CDS <222> (1)..(234) <400> 269 cgc gga gag ggg gag act gag aga ggg cag aat gag gag act cac gca Arg Gly Glu Gly Glu Thr Glu Arg Gly Gln Asn Glu Glu Thr His Ala 10 acg aac aag too toa ggo gto goo agt ttg gag goa coa gog tog tto Thr Asn Lys Ser Ser Gly Val Ala Ser Leu Glu Ala Pro Ala Ser Phe 20 25 Ala Gln Glu Gly Asp Gly Gly Arg Arg Glu Glu Ala Ser Gln Ala Lys atg ggg acg tct tcc ccg tcg aat cag gtg atc aac gtt gta gac gaa 192 Met Gly Thr Ser Ser Pro Ser Asn Gln Val Ile Asn Val Val Asp Glu 50 55 60 gac gag gag gac gac gag gaa gca gag gcg caa gag gca ccc gg 236 Asp Glu Glu Asp Asp Glu Glu Ala Glu Ala Gln Glu Ala Pro 65 70 <210> 270 <211> 78 <212> PRT <213> Toxoplasma gondii <400> 270 Arg Gly Glu Gly Glu Thr Glu Arg Gly Gln Asn Glu Glu Thr His Ala 5 10 Thr Asn Lys Ser Ser Gly Val Ala Ser Leu Glu Ala Pro Ala Ser Phe 20 25

161 .

40

Ala Gln Glu Gly Asp Gly Gly Arg Arg Glu Glu Ala Ser Gln Ala Lys

35

Met Gly Thr Ser Ser Pro Ser Asn Gln Val Ile Asn Val Val Asp Glu
50 55 60

Asp Glu Glu Asp Asp Glu Glu Ala Glu Ala Gln Glu Ala Pro 65 70 75

<210> 271

<211> 423

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(423)

<400> 271

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agt ctg gtt ttg ccc tcc aga ggg gag gag gag gcg aga gag gag acg 96 Ser Leu Val Leu Pro Ser Arg Gly Glu Glu Glu Ala Arg Glu Glu Thr 20 25 30

tct gca acg cgc cag atg ccg acg ctt ctc tct tcg ccg agg cct cca 144
Ser Ala Thr Arg Gln Met Pro Thr Leu Leu Ser Ser Pro Arg Pro Pro
35 40 45

ctc gcg ctg ggg ttg gga gac gag tct ccc tgc gga gag tgg gtg tcg 192 Leu Ala Leu Gly Leu Gly Asp Glu Ser Pro Cys Gly Glu Trp Val Ser 50 55 60

ccg aat gac atg gtt tct gcg ttg tcc ctc tgg gaa gca ggc gag gct 240 Pro Asn Asp Met Val Ser Ala Leu Ser Leu Trp Glu Ala Gly Glu Ala 65 70 75 80

tgg cag ttc aag aca gcg aaa att ctt gac tct ttc gaa ggg gag acc 288
Trp Gln Phe Lys Thr Ala Lys Ile Leu Asp Ser Phe Glu Gly Glu Thr
85 90 95

cca gaa ggg gag gga tgc ggc gca cag gaa aag gac agc cgc atg caa 336 Pro Glu Gly Glu Gly Cys Gly Ala Gln Glu Lys Asp Ser Arg Met Gln 100 105 110

gct ggt gcg act ccc ggt gaa cgt gga ggg gcg gtc gac gaa ggt gtg 384 Ala Gly Ala Thr Pro Gly Glu Arg Gly Gly Ala Val Asp Glu Gly Val 115 120 125

gag ctt ggc tct tct ttc ttc tct gcg tct gaa gat ccg 423
Glu Leu Gly Ser Ser Phe Phe Ser Ala Ser Glu Asp Pro
130 135 140

<210> 272

<211> 141

<212> PRT

<213> Toxoplasma gondii

<400> 272

Arg Gly Ile Pro Asp Gln Arg Ser Ser Arg Ser His Thr Gly Val Glu
1 5 10 15

Ser Leu Val Leu Pro Ser Arg Gly Glu Glu Glu Glu Glu Glu Thr 20 25 30

Ser Ala Thr Arg Gln Met Pro Thr Leu Leu Ser Ser Pro Arg Pro Pro 35 40 45

Leu Ala Leu Gly Leu Gly Asp Glu Ser Pro Cys Gly Glu Trp Val Ser 50 55 60

Pro Asn Asp Met Val Ser Ala Leu Ser Leu Trp Glu Ala Gly Glu Ala 65 70 75 80

Trp Gln Phe Lys Thr Ala Lys Ile Leu Asp Ser Phe Glu Gly Glu Thr 85 90 95

Pro Glu Gly Glu Gly Cys Gly Ala Gln Glu Lys Asp Ser Arg Met Gln
100 105 110

Ala Gly Ala Thr Pro Gly Glu Arg Gly Gly Ala Val Asp Glu Gly Val 115 120 125

Glu Leu Gly Ser Ser Phe Phe Ser Ala Ser Glu Asp Pro 130 135 140

<210> 273

<211> 514

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(513)

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ctg Leu	cct Pro	cct Pro	atg Met 20	Pro	ctg Leu	ccc Pro	gaa Glu	gca Ala 25	Pro	: gaa Glu	gac Asp	ttt Phe	gac Asp 30	Gln	gct Ala	96
cct Pro	atg Met	cca Pro 35	ctg Leu	ccc Pro	gag Glu	gca Ala	ccc Pro 40	gaa Glu	gac Asp	ttt Phe	gac Asp	cag Gln 45	gct Ala	cct Pro	atg Met	144
cca Pro	ctg Leu 50	ccc Pro	gag Glu	gca Ala	ccc Pro	gaa Glu 55	gac Asp	ttt Phe	gac Asp	cag Gln	cct Pro 60	cct Pro	atg Met	cca Pro	ctg Leu	192
ccc Pro 65	gag Glu	gca Ala	ccc Pro	gaa Glu	gac Asp 70	ttt Phe	gac Asp	cag Gln	gct Ala	cct Pro 75	atg Met	cca Pro	ctg Leu	ccc Pro	gaa Glu 80	240
gca Ala	ccc Pro	gaa Glu	gtc Val	ttt Phe 85	gac Asp	cag Gln	gct Ala	cct Pro	atg Met 90	cca Pro	ctg Leu	ccc Pro	gag Glu	gca Ala 95	ccc Pro	288
gaa Glu	gtc Val	ttt Phe	gac Asp 100	cag Gln	gct Ala	cct Pro	atg Met	cca Pro 105	ctg Leu	ccc Pro	gaa Glu	gca Ala	ccc Pro 110	gaa Glu	gac Asp	336
ttt Phe	gac Asp	cag Gln 115	gct Ala	cct Pro	atg Met	cca Pro	ctg Leu 120	ccc Pro	gaa Glu	gca Ala	ccc Pro	gaa Glu 125	gtc Val	ttt Phe	gac Asp	384
cag Gln	gct Ala 130	cct Pro	atg Met	cca Pro	Leu	ccc Pro 135	gag Glu	gca Ala	ccc Pro	gaa Glu	gac Asp 140	ttt Phe	gac Asp	cag Gln	gct Ala	432
cct Pro 145	atg Met	cca Pro	gtg Val	Pro	gag Glu 150	gca Ala	ccc Pro	gaa Glu	gac Asp	ttt Phe 155	gac Asp	cag Gln	gct Ala	cct Pro	gag Glu 160	480
	ctg Leu							Phe			g					514

<210> 274

<211> 171

<212> PRT

<213> Toxoplasma gondii

<400> 274

Arg Asp Gln Ala Ser Met Pro Leu Pro Pro Ala Pro Glu Asp Phe Asp 1 5 10 15

Leu Pro Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala
20 25 30

Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala Pro Met 35 40 45

Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Pro Pro Met Pro Leu 50 55 . 60

Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala Pro Met Pro Leu Pro Glu 65 70 75 80

Ala Pro Glu Val Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro 85 90 95

Glu Val Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Asp 100 105 110

Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Val Phe Asp 115 120 125

Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala 130 135 140

Pro Leu Pro Glu Ala Ala Glu Glu Phe Asp Pro 165 170

<210> 275

<211> 16

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic Primer

WO 99/32633	PCT/US98/27137
<400> 275	
tgcttctcaa aagccg	16
	10
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<210> 276	
<211> 21	
<212> DNA	
<213> Artificial Sequence	
<220>	
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Primer Synthetic	
<400> 276	
cgattgcctg caagaagtgt g	21
<210\ 277	
<210> 277 <211> 20	
<211> 20 <212> DNA	
<213> Artificial Sequence	
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<220>	
<223> Description of Artificial Sequence: Synthetic	:
Primer	
4400- 077	
<400> 277	
acagttttct ccatttcagg	20
<210> 278	
<211> 19	
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Primer Synthetic	
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<211> 17	
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WO 99/32633	PCT/US98/27137
<220>	
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<210> 280	
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gaaaacggtg ccctaaag	18
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<211> 1225	
<212> DNA	
<213> Toxoplasma gondii	
<220>	
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<222> (1)(87)	
<400> 282	
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Gly Arg Val Ser Gln Lys Lys Thr Leu Val Cys Ala Arg Arg Arg	

tct ctt cgg cct ctc gga cga acc gag ttt tca cgg tga accttttgtg 97 Ser Leu Arg Pro Leu Gly Arg Thr Glu Phe Ser Arg

cttacttttc gtctcagact gtgttgttgt tccctgcttc tcaaaagccg cccttccctc 157 acttcttgtt cgccgattgc cttcaagaag tgtggagttc ccttcctttt ttccgggttc 217 tccggaagcc tcctgtagca aaatgcgctg aaattttgga cacttctcga cggtgtctcg 277 ctttaggacg gacctcatgg tctttagggc accgttttct ttcacttttt ctaggaacat 337 cacagttttc tccatttcag ggaacgaaca atctgcaagc gtccacttgt cctgtggctg 397 ctgggctcgg atgccgcctc tgtttagcaa ttgtagcagg caccggatgg caagctaggt 457 ccacttcact gcagtttcaa cttccaaacc aaggcatctc aatttgtatc gtgttctctg 517 tcaacaagct gttgaacctg tcgacggagt gtcgtcccgg ctcctatccc gcgttcgcaa 577 gccgcaccgt tttcagaaca gtgttccccg tggtgttgaa aycgggctgc gaagcgcgag 637 cgtttcgttt tgtggttttt tctgggaaac gatggggatc tcttcgtgtg gcgagacgct 697 tgcctcctgt ttcaaggcgg tgaagtccgg aaccgttgac ttcaaggggc aggagcgagt 757 atactcgtgg ttgatatact ttgcgtgggc gggtggcctc agcgggtttt tcgtcggagg 817 gattctggaa gacttcacgg tcacagtgta cacgatcttg atgtgcatgg ccattgcggc 877 gattetetgt ttteegtegt ggecatgttt ceacagaeae eetgtegagt ggaegeegea 937 cgaccccgcc aggctggctg ctctcttcac gcagcatcaa acccaggaag aaactcctca 997 gaaaggtgcg gggaaaaaac gagggaagaa gagcgctgaa gtgcaacgga aaaactgagt 1057 gtgtgtgcgt atgtagacaa gtgagtcttc ccagagttcg tccggattgt tgcgtggatc 1117 gtgtcaactg gacttctcgt tcgtcaaaga cctggtcgtc tgacccatct gctctccata 1177 1225

<210> 283

<211> 28

<212> PRT

<213> Toxoplasma gondii

<400> 283

Gly Arg Val Ser Gln Lys Lys Thr Leu Val Cys Ala Arg Arg Arg Gln
1 5 10 15

Ser Leu Arg Pro Leu Gly Arg Thr Glu Phe Ser Arg
20 25

<210> 284

<211> 1225

<212> DNA

<213> Toxoplasma gondii

<400> 284

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WO 99/32633		PCT/US9	8/27137
aaggaaggga actccacact tettgaagge aateggegaa o	caagaagtga	gggaag g gcg	1080
gcttttgaga agcagggaac aacaacacag tctgagacga a	aaagtaagca	caaaaggttc	1140
accgtgaaaa ctcggttcgt ccgagaggcc gaagagattg t	ctacgeegg	gcacagacaa	1200
gcgtcttttt ctgcgacact cgacc			1225
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<211> 20			
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<210> 286			
<211> 19		-	
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Primer			
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acacgaagga cctgtatgg		1	9
<210> 287			
<211> 19			
<212> DNA	·		
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<223> Description of Artificial Sequence: Syn	thetic		
Primer	-		
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gtgcttcgat ttgaatgcg		1	9
		_	-

WO 99/32633	PCT/US98/271	3
<210> 288		
<211> 17		
<212> DNA		
<213> Artificial Sequence		
•		
<220>		
<223> Description of Artificial S	Sequence: Synthetic	
Primer		
<400> 288		
tcaaatcgaa gcacatg	17	
<210> 289		
<211> 19		
<212> DNA		
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Primer		
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tttcccagac cttgctgtc	19	
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<211> 20		
<212> DNA		
<213> Artificial Sequence		
12132 Artificial Dequence		
<220>		
<223> Description of Artificial S	Sequence: Synthetic	
Primer	•	
<400> 290		
tcattcaggt ccatcgtcgg	20	
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Primer		

WO 99/32633 PC	T/US98/27137
<400> 291	
cgaggcacaa gtctgcaatg	20
<210> 292	
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<212> DNA	
<213> Toxoplasma gondii	
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<221> CDS	
<222> (1)(222)	
<400> 292	
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Ala Ala Ser Leu Phe Thr Leu Arg Lys Asn Glu Thr Pro Asp Ala G	ai 48
1 5 10 ASP ATA G	LII
gga cga tgt gcg cgg ccg gaa ggt gca gtt ggt ggc ggc ttt cct ga	aa 96
Gly Arg Cys Ala Arg Pro Glu Gly Ala Val Gly Gly Gly Phe Pro Gl	l y
20 25 30	•
set acc act acc acc to the	
ect gee get gea gae tgt gee ttt eac egt ggg gaa gga ega eau ge	144
Pro Ala Ala Ala Asp Cys Ala Phe His Arg Gly Glu Gly Arg Gln Gl	· Y
40 45	
too ggo gtt ott ggo caa gto goo tot ggg gog tot goo co: gtt gg	ıu 192
Ser Gly Val Leu Gly Gln Val Ala Ser Gly Ala Ser Ala Pro Val Gl	192 U
50 55 60	,
gtc cga ggt cgg cgt gtg tct gtt tga aagcaacgcg atttqccqct	242
Val Arg Gly Arg Arg Val Ser Val	
65 70	
tectegegeg acttegegee gaeaagtgee tgtaeggega gaegettgeg ququuqq	200
January and the substitution of the substituti	Tar: 302
aagtggacat gtggttggac ttctcgaccc tcgaagtcga gattccgatg tittgct	taa 362
tgcagggggg aaaggttgcg gagcgcgcgc agagcgacct ggcgcaggca ct 14473	311 422
togacgooca octgaagacg ogcacottoa tggtgggoga gaacatcacc att unag	uct 482
tgtgcctcgt cgcggtgctg agctacggct tccggtccgg	540
	cac 542
tgctcgagaa gcgtccgtac ttgaagcgct tctacgagac cgtggtgaat caquaga	act 602
tcaagaagat cttcggcgag gcgaaggcag cgccacaggc cgccgccaag aaggaga	ctc 662

ccaaagccgc ggcgaagcct gcacagagcg ccggcgatga cgaagaaccg gcgaagaagc 722 ctgcagtcaa gtgcgagttg gacttgctcc cagagccgac gatggacctg aatgagtgga 782 agggcgtgta ctccaacacg aaggacctgt atggcacagc gatgaaatgg ttctgggaac 842 acctcgacgc ggcagggtat tccttgtggt acatgaaata tcagaaactc gagggcgagt 902 geaccytege gttegteace tegaaccage teggeggett cetgeagegg ategaecegg 962 cetteegeaa atacteette ggegtegteg aegtgatggg egagaaegge tgettegaea 1022 tegagggtgt etggetgtte egeggeeaag aegtaeecag ettgatgaag gaeeaecegt 1082 cgtacgagta ccacacttgg cagaaactcg acgtcgccag cgcaaaaagac aagcaactcg 1142 togcagactt ttggtgcgcg tgcgacgaca tccaaggtcg ccccatcgcc gacagcaagg 1202 tctggaaata aaaggaaata acgcacttcg cgaaacgaag gggcggcaac agaggtgtgt 1262 gtctttggtg ctgtgaaaaa aagcgacgcg taaaaaacgg cgagaaatgt tcgtqqcgtg 1322 gcgtgcggtg agaggggtac agtggcgaag ggtcacaaac ccatgtgctt cgatttgaat 1382 gegetteeat etgtacacet ggetetteeg tgegteettt eagtetetee taaaaatete 1442 gtttcacgcg ggtgcagttg cggtacttca ggagcttcqc aggcgccqct cqcqcqcct 1502 ccccgctcta ggaactctca cacgacccca tttatgtcaa ctcgaaaaaa aaaaaaaaa 1562 aaaaaaaaa a 1573

<210> 293

<211> 73

<212> PRT

<213> Toxoplasma gondii

<400> 293

Ala Ala Ser Leu Phe Thr Leu Arg Lys Asn Glu Thr Pro Asp Ala Gln
1 5 10 15

Gly Arg Cys Ala Arg Pro Glu Gly Ala Val Gly Gly Gly Phe Pro Gly 20 25 30

Pro Ala Ala Ala Asp Cys Ala Phe His Arg Gly Glu Gly Arg Gln Gly 35 40 45

Ser Gly Val Leu Gly Gln Val Ala Ser Gly Ala Ser Ala Pro Val Gly
50 55 60

Val Arg Gly Arg Arg Arg Val Ser Val 65 70

<210> 294

<211> 1573

<212> DNA

<213> Toxoplasma gondii

<400> 294

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teeecectge accaageac acateggaat etegacetteg agggtegaga agtecaacea 1260
catgteeact tgteeetget eegeaagegt etegeegtae aggeacttgt eggegegaag 1320
tegegegagg aageggeaaa tegegttget tteaaacaga eacaegeege egacetegga 1380
eteeaacagg ggeagaegee eeagaggega ettggeeaag aaegeeggat eettgtegte 1440
etteeceacg gtgaaaggea eagtetgeag eggeaggtee aggaaageeg eeaeeaactg 1500
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<220>

<223> Description of Artificial Sequence: Synthetic
 Primer

<400> 295

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20

<210> 296

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<212> DNA

<213> Artificial Sequence

<220>

<400> 296

cctactgtga ctcccatcac 20

<210> 297

<211> 19

WO 99/	32633	PCT/US98/27137
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atcaco	cacta agcgtaggg	19
.010.		
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<220>		
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	Primer	
4400	200	
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ccgaaa	gaac gaagetgee	19
<210>	299	
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.000		
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	Description of Artificial Sequence: Synthetic Primer	
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<400>	299	
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	Artificial Sequence	
	•	
<220>		
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                                                                   21
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<223> Description of Artificial Sequence: Synthetic
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<400> 303
acggaagaag acggagatgg
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<211> 20
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<213> Artificial Sequence
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<223> Description of Artificial Sequence: Synthetic
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WO 99/32633	PCT/US98/27137
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212> DNA	
<pre><213> Toxoplasma gondii</pre>	
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221> CDS	
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400> 306	
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cttcgaceg egteegggga etgaggtetg etgteeaege aggaetteet ette	
tegegeege cagaegaggt ettecegaet tttegtetge aegettetee geat	ctccgc 167
tctccccat ctccgtcttc ttccgttttt gactgtctgc gctctttctc cgac	ctcgct 227
gaacgeege gattetegee ttttatttee eegeetegte teeggeegtt eeac	gcacct 287
.cctcggccg ttgcctcgcg ttcctcgggt gtacagacac ctgagcgctc tgcg	ittgtcg 347
agcatttet etgageegea acatggggaa tgegeageet egeggeggag gett	cccagg 407
ggctctgaa gaagacaaga agaaggagag aaagagactt gaagctgcgc ctcc	aacgca 467
eattogalaa agaaagaaga aaggaaaaga ggaggtoggt	

gacteceate accaagtgte geetgegtet geteegacte gagegeataa aagactaeet 587 tettetggag gaagagtata tteteaacea ggageagegg aageeggegg aggagaagaa 647 cgaagaagat gtgaatcgcg tggacgagct ccgtggatca ccactaagcg tagggaatct 707 cgaggaaatc atcgatgaac agcatgcaat cgtttcttcc tccatcggtc ccgagtacta 767 cgtcaacatc ctctctttcg tcgacaaaga cctgctcgag cctggatgca gtgtccttct 827 tcacaacaaa acgagcagca ttgtcggaat tttgaacgac gaggtggacc ctctcatctc 887 ggtcatgaaa gtggagaagg caccgcttga gacgtatgca gacatcggcg gactggagaa 947 gcagattcag gaggtgaagg aggccgtgga atttcctctc acgcatccgg agttcttcga 1007 cgacatcggt atcagecete egaagggtgt cateetetae ggaceeeeg ggacaggaaa 1067 gactetgete gegaaggeeg tggegaacga gacgtegget aegtteette gegtegtegg 1127 aagtgaactc attcaaaaat atttgggaga cggcccgaag ctggtccggg aaatgtttaa 1187 actogotoac gagoacgogo ogagoatogt ottoatogto ttoatogaga gtotagacoo 1247 tgcgctcatt cggcctggac gcattgatcg gaaaattcaa ctccccaatc cggacgcgaa 1307 aaccaagcga aaaatcttcc agatccacac agcgaaaatg accatggccg acgacgtcga 1367 cctcgaggaa tttgttatgg cgaaagacga actgtcgggt gcagatatca aggcgacatg 1427 cacggaggcg gggttgctgg ccttgcgaga gcgacgcatg aaaatcaccc aggaagatct 1487 gcggaaggcg aaggagaagg cgctgtatca gaagaaaggg aacattccag agagtctgta 1547 tctgtgaaga ggcgggagaa aacaatggtg tctccccaga gcgtcgagac agctcgaaga 1607 acgaagetge etetteagea eeeteeagat etgageeagg ageceaacte gatttgtgge 1667 ctcccaagaa ggactgacat ctcgaggcga agaagagact ggaaaatgtc acagcaggct 1727 tcagagacat cttctgctga gaagaaaggc tgtgcgagcg ctttcgactc gtcagttttg 1787 aggcgccgcc ggtagcgcac accetgtggc ttcggtttct ctgtgataga actgtcgaaa 1847 cgctgcgaaa atagaaatga cgaggtcgca ctgcaagaag acaagagaga ttcaaaagaa 1907 aaggttgttt gcccgaaaac ttagcgtctg cgtgtctgat tttttcgagt tgagttgcca 1967

gegegaacce geatgatege ggagtggggg caeteaaage gaaacegttt ategecaaca 2027
gagtgaacceg tteagacett tttteettgge geatgeaaga aaaaagatgt gaactgegta 2087
gtegeacteg gegatteeeg egggtgaggg agtgggagta gegaatteag tegaaagega 2147
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tgttgacage gacaagaaag ceacacaaat gettgggtee acgggagege tgteececag 2327
acacacgeag aaatacgagt gaaacttaet etgetteetg egacaacttt egeaattaeg 2387
agtggaacge tteaaaaaaa aaaaaaaaaa

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<210> 308 <211> 2417 <212> DNA <213> Toxoplasma gondii

<400> 308
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atttgtgtgg ctttcttgtc gctgtcaaca cgatccttcc taaaaaatac acggagaacc 180
tgtgccggcc ctggttcttc tctcaaacag acacgttcgt gactttttc cttcggcaaa 240
tctgtcgcgt ctgcgctctg gaaagcctct tcgcttcga ctgaattcgc tactccact 300
ccctcacccg cgggaatcgc cgagtgcgac tacgcagttc acatctttt tcttgcatgc 360
gccaagaaaa aaggtctgaa cggttcactc tgttggcgat aaacggtttc gctttgagtg 420

ccccactcc gcgatcatgc gtgtccacac tggcaactca actcgaaaaa atcagacacg 480 cagacgetaa gttttcgggc aaacaacett ttettttgaa tetetettgt ettettgeag 540 tgcgacctcg tcatttctat tttcgcagcg tttcgacagt tctatcacag agaaaccgaa 600 gccacagggt gtgcgctacc ggcggcgcct caaaactgac gagtcgaaag cgctcgcaca 660 geetttette teageagaag atgtetetga ageetgetgt gaeattttee agtetettet 720 tegeetegag atgteagtee ttettgggag geeaeaaate gagttggget eetggeteag 780 atctggaggg tgctgaagag gcagcttcgt tcttcgagct gtctcgacgc tctggggaga 840 caccattgtt ttctcccgcc tcttcacaga tacagactct ctggaatgtt ccctttcttc 900 tgatacageg cetteteett egeetteege agatetteet gggtgatttt catgegtege 960 tetegeaagg ceageaacce egecteegtg catgtegeet tgatatetge accegacagt 1020 togtotttog coataacaaa ttootogagg togacgtogt oggocatggt cattttogot 1080 gtgtggatct ggaagatttt tcgcttggtt ttcgcgtccg gattggggag ttgaattttc 1140 cgatcaatgc gtccaggccg aatgagcgca gggtctagac tctcgatgaa gacgatgaag 1200 acgatgeteg gegegtgete gtgagegagt ttaaacattt ceeggaceag ettegggeeg 1260 teteccaaat attittgaat gagtteactt eegacgaege gaaggaaegt ageegaegte 1320 tegttegeea eggeettege gageagagte ttteetgtee eggggggtee gtagaggatg 1380 acaccetteg gagggetgat accgatgteg tegaagaact eeggatgegt gagaggaaat 1440 tecaeggeet cetteacete etgaatetge ttetecagte egeegatgte tgeataegte 1500 tcaagcggtg ccttctccac tttcatgacc gagatgagag ggtccacctc gtcgttcaaa 1560 attecgacaa tgetgetegt tttgttgtga agaaggacae tgeatecagg etegageagg 1620 tctttgtcga cgaaagagag gatgttgacg tagtactcgg gaccgatgga ggaagaaacg 1680 attgcatgct gttcatcgat gatttcctcg agattcccta cgcttagtgg tgatccacgg 1740 agetegteea egegatteae atettetteg ttetteteet eegeeggett eegetgetee 1800 tggttgagaa tatactcttc ctccagaaga aggtagtctt ttatgcgctc gagtcggagc 1860

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agtcgggaag acctcgtctg gcggcgaa aaaggagaag aggaagtcct gcgtggacag 2340
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<210> 309

<211> 20

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic Primer

<400> 309

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<210> 310

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic Primer

<400> 310

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18

20

<210> 311

<211> 1785

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(75)

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atc tgg cag cct ccc gct cat ttt tag tcagcaaaaa tggcacccgc 95

Ile Trp Gln Pro Pro Ala His Phe
20 25

acttgtgcag aggagaaaga aggtggccat gattggctct ggcatgattg gtggcactat 155 gggctacctg tgcgctctcc gtgagctcgc tgacgtcgtt ctctacgatq ttqtcaaagq 215 tatgocogag ggtaaggeto ttgacotgag coatgtgaco teegtggteg acaccaacgt 275 ttccgtccgt gctgagtact cttacgaggc cgcgctcacc ggtgcggact qcqttatcqt 335 taccgccggt ctgaccaagg tgccgggcaa gcccgactcc gagtggagcc quadcqutct 395 gotocogtto aactogaaga toattogoga gatoggtoag aacatoaaga aqtastqsss 455 caagacette ateategtgg teaceaacee getggaetge atggteaagg teatgtgegu 515 ggeototggo gtocogacca acatgatotg oggtatggoo tgcatgotog actitanted 575 ottoogooga taogtogoog acgogotgto tgtototooo ogogacgtoo aniceaseat 635 catoggoaca cacggogact goatggtooc gottgtoogg tacattacog tgaacqacta 695 occgatocag aagttoatoa aggacggogt agtcacggag aagcagotog aggaqutege 755 tgagcacaco aaagtgtotg goggogagat ogtoogotto otoggocagg gricormitis 815 ctacgcccc gccgcatccg ctgtcgccat ggcaacatcc ttcttgaacg acquuaaqcq 875 cgtcatcccg tgcagtgtgt actgcaacgg agagtacggc ttgaaggaca tgttcattgg 935 teteceggee gteattggag gegeeggeat egagegegte ategageteg agetgaacga 995 ggaggagaag aagcagttee agaagteegt egaegaegte atggegetea acaaqqeggt 1055

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tegigittigg acageaaceg egittegetet eaeteaaaac eetigatige agaggggig 1475
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giteegetigea giaetigaaa acaetiggig egeagaggeg agegataggi gegetgaeti 1595
tittitigti tigticaagga aggeatetti tittittita eeggitigtee aetigteatgi 1655
egaaaacgia gieegitga agiggitiga eeeetigtig eetitigteaac aaaaaaaaa 1775
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<210> 312

<211> 24

<212> PRT

<213> Toxoplasma gondii

<400> 312

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<210> 313

<211> 1785

<212> DNA

<213> Toxoplasma gondii

<400> 313

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gcccaaatcg tctgtgttcg aggtgaagat cacatgtccg ctgcagtact gaaaaacact 1560
tggtgcgcag aggcgagcga taggtgcgct gactttttt ttgtttgttc aaggaaggca 1620
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- <210> 314
- <211> 18
- <212> DNA
- <213> Artificial Sequence
- <220>
- <223> Description of Artificial Sequence: Synthetic Primer
- <400> 314
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18

- <210> 315
- <211> 18
- <212> DNA
- <213> Artificial Sequence
- <220>
- <223> Description of Artificial Sequence: Synthetic Primer
- <400> 315
- gatggcatgg atttgacc

18

- <210> 316
- <211> 17
- <212> DNA
- <213> Artificial Sequence
- <220>
- <223> Description of Artificial Sequence: Synthetic Primer
- <400> 316

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<210>				• :
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<220>				

WO 9	9/32633	PCT/US98/27137
<223>	Description of Artificial Sequence: Synthetic	
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	Description of Artificial Sequence: Synthetic	
	Primer	
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cyyact	ticoc cacyatogy	19
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WO 99/32633	PCT/US98/27137											
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<220>												
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<400> 324												
agtcggagaa ggcaccatag 20												
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2220 5												
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Primer	. 57											
<400> 325												
ttcctcctcc ttttcgg	17											
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caa gtc gaa acc atg gag tgc cca act cag	qct qqq caa qca tqc qqc 96											
Gln Val Glu Thr Met Glu Cys Pro Thr Gln												
20 25	30											
aac tat ggt gcc ttc tcc gac tgt gga gtg	cct ctt cgc ggc ttc gcc 144											
Asn Tyr Gly Ala Phe Ser Asp Cys Gly Val	Pro Leu Arg Gly Phe Ala											
35 40	45											
ata aga tta aga aga aga tag aga aga ata	ata and the and and tag. 100											
atg gcc ttc ccc gag aac tgc cca gag ctc Met Ala Phe Pro Glu Asn Cys Pro Glu Leu												
The time the tree time of the tree tree tree tree tree tree tree												

50 55 60

											His				gcc Ala 80	240
										Thr		tac Tyr				288
cgc Arg	tcc Ser	tgc Cys	gat Asp 100	ggc Gly	atg Met	gat Asp	ttg Leu	acc Thr 105	gag Glu	tcc Ser	cgc Arg	ttc Phe	tgc Cys 110	act Thr	ccc Pro	336
												gct Ala 125				384
ggt Gly	tcc Ser 130	ctc Leu	ggc Gly	gag Glu	ttc Phe	ggc Gly 135	gag Glu	tgt Cys	gtg Val	aac Asn	ggc Gly 140	ctt Leu	cag Gln	gag Glu	aga Arg	432
												cag Gln				480
												G] À ààà				528
												aac Asn				576
gcc Ala																624
gag Glu												gag Glu				672
cca Pro 225												cct Pro				720
gag Glu																768

	** (בוכר נ	2000													3370127
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-	_		-		-			-	ggc Gly			-		-		912
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					-				gga Gly 330	-						1008
									gag Glu					-		1056
						-			cat His	_						1104
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									gaa Glu							1296
									gag Glu							1344

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														gtc Val		1536
														gca Ala		1584
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														gtc Val		1680
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cccc	agag	itt t	cctg	jttt	c ct	tacc	tccg	g cgt	gcca	tct	tgtt	tttc	gc c	cgcg	cggat	2086
gtgt	taat	gc g	ataa	ataa	c ta	atga	agca	agg	ttat	gaa	cago	ttcg	ıtg a	gcta	tattc	2146

PCT/US98/27137

WO 99/32633

<210> 327

<211> 571

<212> PRT

<213> Toxoplasma gondii

<400> 327

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Gln Val Glu Thr Met Glu Cys Pro Thr Gln Ala Gly Gln Ala Cys Gly
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Asn Tyr Gly Ala Phe Ser Asp Cys Gly Val Pro Leu Arg Gly Phe Ala 35 40 45

Met Ala Phe Pro Glu Asn Cys Pro Glu Leu Val Ala Phe Ala Ala Cys 50 55 60

Asp Ala Pro Ala Pro Pro Gln Glu Asp Arg Cys His Ser Phe Ser Ala 65 70 75 80

Trp Ser Lys Cys Thr His Ile Pro Gly Thr Thr Leu Tyr Glu Gln Thr 85 90 95

Arg Ser Cys Asp Gly Met Asp Leu Thr Glu Ser Arg Phe Cys Thr Pro 100 105 110

Asp Glu Glu Val Gly Ser Asp Val Ser Thr Asp Val Ala Ser Glu Cys 115 120 125

Gly Ser Leu Gly Glu Phe Gly Glu Cys Val Asn Gly Leu Gln Glu Arg 130 135 140

Ser Tyr Ser Asp Cys Pro Asp His Lys Glu Val Arg Gln Cys Ser Asp 145 150 155 160

Glu Ser Cys Ser Ala Phe Gly Glu Trp Ser Pro Cys Gly Glu Pro Gln 165 170 175

Gln Gly Leu Arg Ile Arg Lys Arg Arg Ala Cys Asp Asn Val His Cys 180 185 190

Ala Cys Val Glu Ala Glu Val Cys Gly Asp Val Thr Pro Glu Ile Glu 195 200 205

Glu Glu Glu Gly Glu His Phe Pro Pro Glu Glu Gly Glu Val Leu Pro 210 215 220

- Pro Tyr Glu Glu Gly Pro Gly Glu Gly Glu Leu Val Pro Pro Glu Glu 225 230 235 240
- Glu Ile Pro Glu Gly Glu His Val Pro Glu Glu Glu Ile Pro Glu Gly
 245 250 255
- Glu His Ile Pro Glu Glu Leu Pro Glu Gly Glu His Val Pro Glu Glu 260 265 270
- Glu Ile Pro Glu Gly Glu His Val Pro Glu Glu Glu Ile Pro Glu Gly 275 280 285
- Glu His Val Pro Glu Glu Phe Pro Glu Gly Glu His Val Pro Glu Glu 290 295 300
- Glu Ile Pro Glu Gly Glu His Val Pro Glu Glu Glu Ile Pro Glu Gly 305 310 315 320
- Glu His Val Pro Glu Glu Phe Pro Glu Gly Glu His Ile Pro Glu Glu 325 330 335
- Leu Pro Glu Gly Glu His Ile Pro Glu Glu Phe Pro Glu Gly Glu His 340 345 350
- Ile Pro Glu Glu Leu Pro Glu Gly Glu His Val Pro Glu Glu Glu Ile 355 360 365
- Pro Glu Gly Glu His Ile Pro Glu Glu Phe Pro Glu Gly Glu His Val 370 380
- Pro Glu Glu Glu Ile Pro Glu Gly Glu His Ile Pro Glu Glu Glu Phe 385 390 395 400
- Pro Glu Gly Glu His Val Pro Glu Glu Glu Ile Pro Glu Gly Glu His
 405 410 415
- Val Pro Glu Glu Leu Pro Gly Gly Glu Leu Ile Pro Glu Glu Glu 420 425 430
- Ile Pro Glu Gly Glu His Val Pro Glu Glu Leu Pro Glu Gly Glu His
 435
 440
 445
- Val Pro Glu Glu Glu Ile Pro Glu Gly Glu His Val Pro Glu Glu Glu 450 455 460

Ile Pro Glu Gly Glu His Val Pro Glu Glu Glu Thr Pro Glu Gly Glu 465 470 475 480

- His Ala Pro Glu Glu Glu Thr Pro Ala Pro Glu Glu Thr Glu Lys Glu
 485 490 495
- Glu Glu Glu Gly Val Pro Val Ala Ala Ile Ala Gly Gly Val Val Gly
 500 505 510
- Gly Val Leu Leu Ile Ala Gly Gly Ala Gly Ala Ala Val Tyr Ala Asn 515 520 525
- Gln Gly Gly Val Glu Ala Ala Glu Asp Glu Val Met Phe Glu Ser Glu 530 535 540
- Glu Asp Gly Thr Cln Ala Gly Glu Asn Arg Glu Ser Glu Thr Val Ile 545 550 555 560
- Glu Ile Glu Asp Asp Ala Trp Ala Asp Met Asp 565 570

<210> 328

<211> 2167

<212> DNA

<213> Toxoplasma gondii

<400> 328

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cacctgcacc accagcaatg agcaacacac ctccgacgac accaccggca atcgctgcga 660 ctggcacgcc ttcttcctcc tccttttcgg tctcctcagg tgcaggagtc tcttcctctg 720 gagcatgttc teetteaggg gteteeteet caggaacatg etegeettea gggattteet 780 cttcaggaac atgctctcct tcagggatct cctcctcagg aacatgctcg ccttcaggga 840 gctcttcagg aacatgctct ccttcaggga tctcctcctc aggaataagt tctcctccag 900 ggageteete eteaggaaca tgetegeett cagggatete eteeteagga acatgetete 960 cttcagggaa ctcctcctca ggaatatgtt ctccttcagg gatttcctcc tcaggaacat 1020 getegeette tgggaactet teaggaatat geteteette agggatetee teeteaggaa 1080 catgetegee tteagggage teeteaggaa tatgetetee tteagggaac tetteaggga 1140 tatgctcgcc ttcagggagc tcctcaggaa tatgctctcc ttcagggaac tcttcaggaa 1200 catgetetee tteagggatt teetetteag gaacatgtte teetteaggg attteeteet 1260 caggaacatg ctcgccttct gggaactctt caggaacatg ctctccttca gggatttcct 1320 cttcaggaac atgttctcct tcagggattt cctcctcagg aacatgctcg ccttctggga 1380 gctcttcagg aatatgttct ccttcaggga tttcctcctc aggaacatgt tctccttcag 1440 ggateteete etegggagga acaageteae eeteaceagg accetettea tatggaggea 1500 agacctcgcc ttcttcaggg gggaaatgtt cgccttcttc ctcctcaatc tctggggtga 1560 categoegea gaceteggee tegacacagg egeagtgeae gttgtegeat geaegtetet 1620 tgcggatacg caggcettge tggggttece egcagggtga ecaetegeeg aaggeagage 1680 aggattegte agageactga eggaetteet tatgateggg geagteegag tagettetet 1740 cctgaaggcc gttcacacac tcgccgaact cgccgaggga accgcattcg gaagcgacgt 1800 cagtggaaac gtccgagccg acctcctcgt cgggagtgca gaagcgggac tcggtcaaat 1860 ccatgccatc gcaggagcgc gtctgctcgt acagagtagt gccgggtatg tgtgtgcact 1920 tggaccaggc cgagaaagaa tggcagcggt cctcttgggg aggcgcggga gcatcgcaqq 1980 cggcgaaggc cacgagctct gggcagttct cggggaaggc catggcgaag ccgcgaagag 2040

gcactccaca gtcggagaag gcaccatagt tgccgcatgc ttgcccagcc tgagttgggc 2100
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aga ctc acc tca gca gcc gct act gtg tgg acc tcc cag aca agg ttg 96
Arg Leu Thr Ser Ala Ala Ala Thr Val Trp Thr Ser Gln Thr Arg Leu
20 25 30

agt cga tca cct gcg aag tcg gct ccc tgc ccc age ccc cga ctg tcg 144
Ser Arg Ser Pro Ala Lys Ser Ala Pro Cys Pro Ser Pro Arg Leu Ser
35 40 45

ccc ccg gcg agg gtg gtg agg aag acg gca acg cat gtg gac cgt ggg 192
Pro Pro Ala Arg Val Val Arg Lys Thr Ala Thr His Val Asp Arg Gly
50 55 60

tcc gtg gag ccc gtg tcc cgg cga agg aaa caa cat gag cac ccg ccg 240 Ser Val Glu Pro Val Ser Arg Arg Lys Gln His Glu His Pro Pro 65 70 75 80

gag aga aga cac cga gag cga ggc gtg cgt caa cca agt cga aac cat 288 Glu Arg Arg His Arg Glu Arg Gly Val Arg Gln Pro Ser Arg Asn His 85 90 95

gga gtg .ccc aac tca ggc tgg gca agc atg cgg caa cta tgg tgc ctt 336 Gly Val Pro Asn Ser Gly Trp Ala Ser Met Arg Gln Leu Trp Cys Leu 100 105 110

ctc cga ctg tgg aat gcc tct tcg cgg ctt cgc cat ggc ctt ccc cga 384
Leu Arg Leu Trp Asn Ala Ser Ser Arg Leu Arg His Gly Leu Pro Arg
115 120 125

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														gtg Val		480
														cga Arg 175		528
														ggt Gly		576
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acat	ttcc	cc c	ctga	agaa	g gc	gagg	tctt	gcc	tcca	tat	gaag	aggg	tc d	etgat	gagag	924
tgag	cttg	tt c	ctcc	cgag	g ag	gaga	tccc	tga	agga	gaa	catg	ttcc	tg a	aggaa	qaaat	984
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tgaa	gagg	aa a	tccc	tgaa	g ga	gaac	atgt	tcc	tgaa	gag	ttcc	c+ga	ag d	์ เลิสลิล	catut	1224
tect	gagg	ag c	etece	tgaa:	g ga	ıgago	atat	. tca	tgaa	gag	ttcc	ctga	ag ç	gugad	catat	1284
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tatt	cctg	ag ç	gagtt	ccct	g aa	ggcg	agca	ı tgt	tcct	gag	gago	jaaat	cc d	etgaa	ggaga	1404
acat	atto	ct c	gagga	ıggag	t to	cctg	jaagg	, aga	gcat	gtt	ccto	gagga	gg a	agato	cctda	1464

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<210> 330

<211> 197

<212> PRT

<213> Toxoplasma gondii

<400> 330'

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Arg Leu Thr Ser Ala Ala Ala Thr Val Trp Thr Ser Gln Thr Arg Leu
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Ser Arg Ser Pro Ala Lys Ser Ala Pro Cys Pro Ser Pro Arg Leu Ser 35 40 45

Pro Pro Ala Arg Val Val Arg Lys Thr Ala Thr His Val Asp Arg Gly 50 55 60

Ser Val Glu Pro Val Ser Arg Arg Arg Lys Gln His Glu His Pro Pro 65 70 75 80

Glu Arg Arg His Arg Glu Arg Gly Val Arg Gln Pro Ser Arg Asn His 85 90 95

Gly Val Pro Asn Ser Gly Trp Ala Ser Met Arg Gln Leu Trp Cys Leu 100 105 110

Leu Arg Leu Trp Asn Ala Ser Ser Arg Leu Arg His Gly Leu Pro Arg 115 120 125

Glu Leu Pro Arg Ala Arg Gly Leu Arg Arg Leu Arg Cys Ser Arg Ala 130 135 140

Ser Pro Arg Gly Pro Leu Pro Phe Phe Leu Gly Leu Val Gln Val His 145 150 155 160

Thr His Thr Arg His Tyr Ser Val Arg Ala Asp Ala Leu Leu Arg Trp.
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His Gly Phe Asp Arg Val Pro Leu Leu His Ser Arg Arg Gly Gly Arg 180 185 190

Leu Gly Arg Phe His 195

<210> 331

<211> 2392

<212> DNA

<213> Toxoplasma gondii

<400> 331

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ggaaaacagg aaactetggg gaaaacgget tteeacaagg aggeegeagt aggettactg 180
tetaeagegg ettgtgacae aegaagetge ggeageetat atataagetg etggaaaaga 240
tacaccagtg gaaaacgtaa aataaaatgg eaegtgatea agaaagageg gaeggacaea 300

aatggctgcg ttcgtctggt acctagctca gagtgtcacg ccgcctcccc ttcgaaggac 360 aqcegcagac getecgeeg taacacagaa tacetegega teaeggtttt gtegeaegee 420 tqcatqtqcc cacacagact actagtctac tttagtccat gtctgcccat gcgtcatctt 480 eqateteaat gaeegteteg etetegeggt tetegeeage etgggtteeg tettettege 540 totcaaacat cacttogtot toagotgott caacgocaco ttggtttgcg tacacggcag 600 cacctgcacc accagcaatg agcaacacac ctccgacgac accaccggca atcgctgcga 660 ctggcacqcc ttcttcctcc tccttttcgg tctcctcagg tgcaggagtc tcttcctctg 720 qaqcatqttc tccttcaggg gtctcctcct caggaacatg ctcgccttca gggatttcct 780 cttcaggaac atgctctcct tcagggatct cctcctcagg aacatgctcg ccttcaggga 840 qctcttcagg aacatgctct ccttcaggga tctcctcctc aggaataagt tctcctccag 900 qqaqctcctc ctcaggaaca tgctcgcctt cagggatctc ctcctcagga acatgctctc 960 cttcagggaa ctcctcctca ggaatatgtt ctccttcagg gatttcctcc tcaggaacat 1020 qctcqccttc agggaactcc tcaggaatat gttctccttc agggatctcc tcttcaggaa 1080 catqctcqcc ttcaqqqaqc tcctcatqaa tatqctctcc ttcaqqqaac tcttcatqaa 1140 tatgetetee tteagggage teeteaggaa tatgttetee tteagggaae tetteaggaa 1200 catgitetee ticagggatt tecteticag gaacatgite tecticaggg atticetet 1260 caggaacatg ttcgccttct gggaactctt caggaacatg ctctccttca gggatttcct 1320 cttcaggaac atgttctcct tcagggattt cctcctcagg aacatgctcg ccttctggga 1380 gctcttcagg aatatgttct ccttcaggga tttcctcctc aggaacatgt tctccttcag 1440 ggatetecte etegggagga acaageteae eeteaceagg accetettea tatggaggea 1500 agacctegee ttetteaggg gggaaatgtt egeettette etecteaate tetggggtga 1560 categorgea gaceteggee tegacacagg egeagtgeae gttgtegeat geaegtetet 1620 tgcqqatacq caqqccttqc tggggttccc cgcagggtga ccactcgccg aaggcagagc 1680 aggattegte agageactga eggaetteet targateggg geagteegag tagettetet 1740

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<211> 21

<212> DNA

<213> Artificial Sequence

<220>

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Primer

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<210> 333

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
Primer

<400> 333

tcttaaagcg ttcgtggtc 19

<210>	334	·		
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<400> 337

agtcagaagc agtcaaggc 19

<210> 338

<211> 647

<212> DNA

<213> Toxoplasma gondii

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<213> Toxoplasma gondii

<400> 339

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tctccccgac tctgaacggg cggtttcgga tgtctttggc ttcttcgtat ttgctgcac 480
gacacaacgg catgtccaca cgtgacgtga acagtgaaca aagaaccggc ccacgcttgt 540
agacgataaa attttcacg ggatgtggct tctttgagct gtgtagtagt gacactgaca 600
ctatctccga gcaagtctgg ggaatcggtc taagtggcta aaggatc 647

<210> 340

<211> 867

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(867)

<400> 340

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gcg ggc cct gtc tcc cag ctt gct cgg gcg agc gac gac agc gtc gac 96
Ala Gly Pro Val Ser Gln Leu Ala Arg Ala Ser Asp Asp Ser Val Asp
20 25 30

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gaa gag atg cac gag gcc tac gac cct ttg ttg gaa ttc gtt gag acg 192 Glu Glu Met His Glu Ala Tyr Asp Pro Leu Leu Glu Phe Val Glu Thr

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gat gcg atc gac cgc gtg tcc cag ttc gat ctg gtt tcc ctc cta gat 288
Asp Ala Ile Asp Arg Val Ser Gln Phe Asp Leu Val Ser Leu Leu Asp
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			atc Ile					_		-	_	-	_	-	384
			gct Ala								_	-			432
			act Thr							_	_		-		480
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		-	aca Thr									-			624
			act Thr				-					-			672
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Phe Arg Glu Ile Lys Lys Ala Val Glu Glu Asp Ala Ala Leu Ser Thr 65 70 75 80

Asp Ala Ile Asp Arg Val Ser Gln Phe Asp Leu Val Ser Leu Leu Asp 85 90 95

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Lys Thr Thr Thr Thr Ser Ser Ser Thr Ser Thr Ser Thr Thr Thr 145 150 155 160

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Thr Thr Thr Thr Thr Thr Thr Thr Thr Pro Thr Thr Thr Thr

195 200 205

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Ala Gly Pro Val Ser Gln Leu Ala Arg Ala Ser Asp Asp Ser Val Asp
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agc gtc gaa acc gcg cgt cag cac atg gag ctg gct atc gag gct gac 381 Ser Val Glu Thr Ala Arg Gln His Met Glu Leu Ala Ile Glu Ala Asp 35 40 45

gaa gag atg cac gag gcc tac gac cct ttg ttg gaa ttc gtt gag acg
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		agc Ser					_	-	•	621
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		acc Thr 150				-				717
		act Thr				_				765
		aca Thr								813
		acc Thr					_			861
		cca Pro					_			909
		aca Thr 230								9 57
		aca Thr								1005
		act Thr	-	-	-	_				1053
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Glu Glu Met His Glu Ala Tyr Asp Pro Leu Leu Glu Phe Val Glu Thr 50 55 60

Phe Arg Glu Ile Lys Lys Ala Val Glu Glu Asp Ala Ala Leu Ser Thr 65 70 75 80

Asp Ala Ile Asp Arg Val Ser Gln Phe Asp Leu Val Ser Leu Leu Asp 85 90 95

Val Ile Arg Glu Ala Ala Gln Ala Lys Phe Asp Leu Leu Gly Arg Leu
100 105 110

Ile Thr Asp Ile Ala Ser Gly Ile Gly Glu Gly Ala Met Ala Leu Met
115 120 125

Gly Glu Glu Ala Ala Phe Ile Arg Pro Arg Arg Ser Lys Arg Gly Lys 130 135 140

Lys Thr Thr Thr Thr Ser Ser Ser Thr Ser Thr Ser Thr Thr Thr 145 150 155 160

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gtcgttgtgc ttgtagttgt ggttggttcc gtggttgttg ttgtagttgt tggttccgtg 420

gttgtcgttg ttgtagttgg tgtagtagta gttgtggtag tcgtagtggt tgtcgtagat 480 qtcqttqtaq ttgqcqttqt ggttqttqtc gttqttqttg gtqtaqttqt tgtqqttqtt 540 gtcgttgttg ttggtgtagt tgttgtggtt gttgtcgttg ttgttggtgt agttgttgtg 600 qttqttqtag ttqttqttqg tqtcqtaqtt qtaqtaqtaq tcqtaqtqqt gqtaqtqqta 660 gtagtggtag ttgatgtcgt ggtcgttgta ctcgtacttg tggatgaact ggttgtagtt 720 gtagtetttt teeetetett tgaeeteett ggeetaatga aegeageete eteteeeate 780 agagecatgg caccetegee gatteegetg gegatgtetg taatgaggeg teegaggaga 840 tegaaetttg ettgtgeage eteteggatg acatetagga gggaaaceag ategaaetgg 900 gacacgeggt egategeate tgtacteaga geegeatett eeteaacage tittitgatt 960 tecegaaaeg teteaaegaa tteeaaeaaa gggtegtagg cetegtgeat etettegtea 1020 geetegatag ceagetecat gtgetgaege geggtttega egetgtegae getgtegteg 1080 ctcgcccgag caagctggga gacagggccc gccgccacag acagcaccac caaaaacaac 1140 gccgcctgcc ttcctgccat attgcacaaa aaagaagcgc ctttgcgttt ctacgttgca 1200 agaagtettt gettetteag gteagteata cacagageea gegacegeeg taetgaaace 1260 aaacactaga ctttgctcgt ccgtgagagg ttctcgacgt tggcaaaagg gaaaaaaaag 1320 tcttgtgacg gcggaatgct caatgcagtc gaggagcgct tccaggcgcc acagcggcag 1380 1397 ggatatctgg aatgggg

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ational Application No

PCT/US 98/27137 CLASSIFICATION OF SUBJECT MATTER PC 6 C12N15/30 C07K A. CLASS C07K14/45 C12N15/11 C12N5/10 CO7K16/20 A61K39/395 C12Q1/68 A61K48/00 A61K39/002 According to International Patent Classification (IPC) or to both national classification and IPC B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) C12N C07K A61K C12Q Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practical, search terms used) C. DOCUMENTS CONSIDERED TO BE RELEVANT Citation of document, with indication, where appropriate, of the relevant passages Retevant to claim No. Category ' 39 - 42EP 0 516 381 A (MERCK & CO INC) X 2 December 1992 see examples 3,4,6 EP 0 687 471 A (BAYER CORPORATION) 1 - 38Α 20 December 1995 see the whole document EP 0 710 724 A (AKZO NOBEL N. V.) 1-38 Α 8 May 1996 see page 7 - page 9 EP 0 700 991 A (BAYER CORPORATION) 1-38 Α 13 March 1996 see the whole document X Further documents are listed in the continuation of box C. Patent family members are asled in arrest Special categories of cited documents: "T" later document published after the international filing date are rocument published after the appearance of priority date and not its conflict with the appearance but cited to understand the principle or theory underlying the "A" document defining the general state of the lart which is not considered to be of particular relevance invention "E" earlier document but published on or after the international "X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another involve an inventive step when the document is taken alone "Y" document of particular relevance, the claimed invention citation or other special reason (as specified) cannot be considered to involve an inventive step when the document is combined with one or more other, such docu-"O" document referring to an oral disclosure, use, exhibition or ments, such combination being obvious to a person skilled in the art.

19 April 1999

03/05/1999

"&" document member of the same patent family

Date of mailing of the international search report

Name and mailing address of the ISA

Date of the actual completion of the international search

Authorized officer

European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016

document published prior to the international filing date but later than the priority date claimed

Mandl, B

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other means

Int. stional Application No
PCT/US 98/27137

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Inte stional Application No
PCT/US 98/27137

ategory '	ation) DOCUMENTS CONSIDERED TO BE RELEVANT Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	EMBL database entry TGAA19977; accession number AA519977; 17. July 1997; Marra et al.: 'The WashU-Stanford-PAMF-NIH Toxoplasma EST project.' XP002100359 see abstract	1-7,10, 12-31
X	EMBL database entry TGAA20348; accession number AA520348; 17. July 1997; Marra et al.: 'The WashU-Stanford-PAMF-NIH Toxoplasma EST project.' XP002100360 see abstract	1-7,10, 12-31
X	EMBL database entry TG0292; accession number N82029; 13. April 1996; Hehl et al.: 'The WashU-Merck-Stanford-NIH Toxoplasma EST project.' XP002100361 see abstract	1-7,10, 21-31
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X	EMBL database entry TG1673; accession number N82167; 13. April 1996; Hehl et al.: 'The WashU-Merck-Stanford-NIH Toxoplasma EST project.' XP002100363 see abstract	1-7,10, 12-31
X	EMBL database entry TG5032; accession number N81503; 13. April 1996; Hehl et al.: 'The WashU-Merck-Stanford-NIH Toxoplasma EST project.' XP002100364 see abstract	1-7,10, 12-31
X ·	EMBL database entry TGAA20213; accession number AA520213; 17. July 1997; Marra et al.: 'The WashU-Stanford-PAMF-NIH Toxoplasma EST project.' XP002100365 see abstract	1-7,10, 12-31

international application No.

PCT/US 98/27137

Box I	Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)
This Inte	ernational Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1. X	Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely: Remark: Although claims 9 and 32-38 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2.	Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3.	Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II	Observations where unity of invention is lacking (Continuation of item 2 of first sheet)
This Inte	rnational Searching Authority found multiple inventions in this international application, as follows:
1.	As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2.	As all searchable claims could be searched without effort justifying an additional fee, this Authority and not invite payment of any additional fee.
3.	As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4.	No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Remark	The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.

Information on patent family members

Inte. Jonal Application No PCT/US 98/27137

Patent document cited in search report		Publication date		Patent family member(s)		Publication date
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WORLD INTELLECTUAL PROPERTY ORGANIZATION International Bureau



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US

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(54) Title: TOXOPLASMA GONDII PROTEINS, CORRESPONDING NUCLEIC ACID MOLECULES, AND USES THEREOF

(57) Abstract

The present invention relates to immunogenic Toxoplasma gondii proteins, to T. gondii nucleic acid molecules, including those that encode such proteins and to antibodies raised against such proteins. The present invention also includes methods to obtain such proteins, nucleic acid molecules and antibodies. Also included in the present invention are compositions comprising such proteins, nucleic acid molecules and/or antibodies, as well as the use of such compositions to inhibit oocyst shedding by cats due to infection with T. gondii. The present invention also includes the use of certain T. gondii-based antisera to identify such nucleic acid molecules and proteins, as well as nucleic acid molecules and proteins identified by such methods. The present invention also relates to methods for the detection of cysts and oocysts.

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